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THE INHERITANCE OF THE WHITE BURLEY CHARACTER IN TOBACCO[1]

By F. S. HENIKA[2]

Research Assistant in Horticulture, Wisconsin Agricultural Experiment Station

INTRODUCTION

Progress in the development of improved strains of tobacco is limited by a lack of exact knowledge relative to the inheritance of specific characters which make up the varietal complex. In the case of the White Burley variety, the basic character is an apparent reduction in chlorophyll, which renders it economically important. Any attempt, therefore, at the improvement of this variety by hybridization must take into account the inheritance of this character. The present study was, in part, prompted by this consideration; for, although White Burley tobacco has been the subject of some genetic investigation in the past, the inheritance of the "white" character has remained obscure.

White Burley tobacco originated in 1865, presumably as a mutation from the green-colored variety Little Burley (Mathewson (*10*)).[3] In appearance, White Burley seedlings are characteristically somewhat lighter green in color than seedlings of green varieties. The stems of White Burley seedlings in particular are clear white and have a glossy appearance in contrast to the dull greenish-white stems of green varieties.

For a few weeks after the seedlings are transplanted, under good growing conditions the color difference between White Burley and green[4] varieties becomes less distinct, especially while the plants are growing rapidly. With the approach of maturity, however, White Burley tobacco loses much of its green color, particularly in the lower leaves and in the stem. If the usual commercial practice is followed, and the plants are topped by breaking off the stem several nodes below the seed head, the loss of chlorophyll in white Burley plants is increased, and within a short time the plants become light yellow in color. Except for a slight mottling of the leaves as they ripen, green varieties retain their color under this treatment.

REVIEW OF LITERATURE

A survey of the literature reveals that in almost every plant genus which has been subjected to intensive genetical investigation, heritable chlorophyll deficiencies have been reported, and in many cases a

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[3] Reference is made by number (italic) to Literature Cited, p. 493.

[4] The term "green" as used in this paper refers to the normal genetic green color of leaves and stalks, and "white" refers to the white or yellow color characteristic of White Burley tobacco.

satisfactory genetic analysis has been made. In the genus Nicotiana four cases may be cited.

Lodewijks (*8*) reported the occurrence and behavior of certain "aurea" forms of *Nicotiana tabacum* which originated as mutations in his experimental fields at Klaten, Java, in 1908 and 1909. Two self-pollinated aurea plants gave rise to two aurea groups, similar in appearance but apparently distinct in genetic behavior. Several self-pollinated generations of group 1 aureas yielded 75 per cent aurea and 25 per cent green. Aureas of group 2, however, yielded 35 per cent aurea and 65 per cent green. Reciprocal crosses between the aureas and the green types made by Lodewijks gave the following results: In group 1, aurea × green yielded 83 per cent aurea in F_1, and the reciprocal cross, green × aurea, 48 per cent aurea. For group 2, crosses with aurea as the female parent gave in F_1 48 per cent aurea, and green × aurea gave 43 per cent aurea.

Since he was unable to establish true breeding aureas of either group, Lodewijks concluded that his aureas, like certain of Baur's Antirrhinums (*2*), existed only in the hybrid form.

Lubimenko and Palamartchouck (*9*) studied the different amounts of chlorophyll present in certain Russian and American varieties of *Nicotiana tabacum* as determined by chemical analysis. Data for the parents and the F_1 of a series of crosses were reported, but no attempt was made at factorial analysis in F_2.

Allard (*1*) studied an aurea form of *Nicotiana rustica*. In crosses with the green form of this species he found that the aurea form behaved as a simple Mendelian recessive.

Kajanus (*5*), working in Sweden, reported the results of crossing White Burley tobacco obtained from Virginia, with a green variety native to the Netherlands. The F_1 of this cross was green, like the green parent, and in F_2 the following distribution was obtained:

	Green	White
Observed	5,037	229
Expected (15 : 1)	4,937	329 $d=100\pm11.85$ [5]

In the F_3 generation, although numerical ratios are not given, constant green, constant white, and segregating families were obtained with the truebreeding green progenies constituting the majority.

In the first three cases cited, the chlorophyll-deficient characters under consideration can not be regarded as being identical with the White Burley character, although they are apparently similar in appearance. The aurea character of Lodewijks is, in fact, quite different since it behaved as a dominant and upon self-pollination gave rise to progenies segregating for aurea and green.

The aurea character reported by Allard (*1, p. 234*) in *Nicotiana rustica* is essentially similar to White Burley in appearance but is a monohybrid and in a different species. The crosses of Lubimenko and Palamartchouck were not carried through the second generation and consequently do not show the number of genetic factors involved.

Only the paper of Kajanus deals with the White Burley character. Since his White Burley variety was obtained from Virginia, it may be safely considered as belonging to the same chlorophyll-deficient group as the varieties used in the present investigation. The evidence of a simple dihybrid relationship between the green and white varieties used by Kajanus is, however, not conclusive.

[5] Application of probable error made by the writer from Kajanus' data.

MATERIALS AND METHODS

Dr. James Johnson laid the foundation for the present investigation in 1916 while engaged in tobacco investigations at the Wisconsin Agricultural Experiment Station. In the course of studies on the inheritance of disease resistance in tobacco he (*4*) crossed a number of green lines with pure lines of White Burley. In subsequent generations from these crosses segregation for color of plant was observed and recorded so that by 1928, when the writer became interested in this material, a substantial body of data dealing with the inheritance of the chlorophyll deficiency had been accumulated.

VARIETIES USED

The majority of Johnson's observations were made on progenies of the cross Little Dutch × White Burley, strain Judy's Pride. Since seed of the F_1, F_2, and F_3 generations from this cross were available as well as seed of the F_1 backcrossed to White Burley, it seemed advisable to use this material in the further analysis of the White Burley character. Little Dutch, the green parent in this cross, is an Ohio strain grown for cigar-filler tobacco in that State.

Judy's Pride is a typical strain of stand-up White Burley tobacco such as is grown extensively in Kentucky. Under optimum conditions it makes a very vigorous growth.

Other green and white crosses made during the course of the investigation involved the following pure-line varieties: SB9AAX, a stand-up White Burley strain resistant to black root rot; Havana 142, a green line of cigar-binder tobacco extensively grown in Wisconsin; Xanthia, a green Turkish strain with small oval leaves; 180A31, a green strain of cigar-binder tobacco; and White Burley, strain Judy's Pride.

FIELD METHODS

In general, the procedure in these earlier investigations was that common to commercial tobacco growing in Wisconsin. Seed was sown in steam-sterilized seed beds early in April, and the seedlings transplanted to the field in June. No conscious selection was exerted in the choosing of plants from the seed bed except as in commercial practice—that is, the earliest plants, and therefore usually the largest plants of an even size, were taken. Counts on segregating progenies were made after topping, when the phenotypic difference between genetically green and genetically white individuals was greatest. In some cases the plants left in the seed beds were permitted to grow until they could be classified; in certain other cases, after field planting the majority of plants were removed from the seed bed and discarded, permitting those remaining to grow until their character could be determined.

METHODS USED IN GREENHOUSE TESTS

With the field method the size of populations was limited by available land and by facilities for culture; consequently other methods for the study of the white character in larger numbers were sought.

Early in 1929 a method was devised for the identification of the chlorophyll deficiency in the seedling stage in the greenhouse. It was found that pure-line green and pure-line White Burley plants gave a different reaction to a period of total darkness at high temperature.

The chlorophyll-deficient character of White Burley seedlings was markedly accentuated by the treatment, whereas pure-line green seedlings were only slightly affected. Experiments with both pure-line and segregating material led to the adoption of a standard etiolation period of seven days at a temperature of 90° F. for seedlings approximately 4 inches in height.

The effectiveness of the etiolation period was governed, apparently, by two factors—temperature and the physiological state of the plants as evidenced by their rate of growth. At temperatures of 50° to 60° F., 14 or 15 days of total darkness were required to etiolate White Burley seedlings completely, as compared with 7 days at 90°. Furthermore, plants not in a state of rapid growth were much less uniform

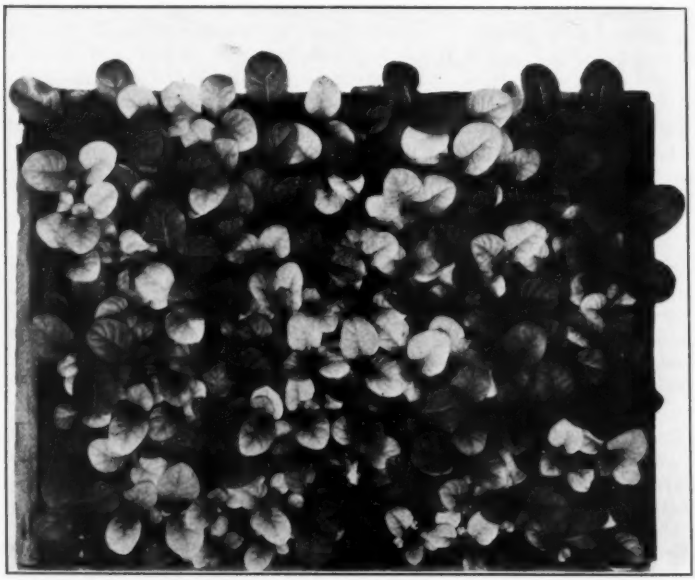

FIGURE 1.—A typical flat of seedlings after etiolation; progeny 63-22♂×W. B.♀ 65 green, 55 white

in their reaction to the etiolation. For this reason, during the darker winter months of December and January, artificial light was used to prolong the daily growing period.

The accuracy of this method was thoroughly checked by experiment with pure green and pure white lines in the greenhouse. In the summer of 1929 a quantity of material that had been classified by the etiolation method was transplanted to the field. Reclassification of this material under field conditions showed that no errors had been made.

Analysis of progenies from various crosses was begun in November, 1929. Seed was sown broadcast in stock flats on soil steam-sterilized for 30 minutes at 98° to 100° C., and seedlings were transplanted to unsterilized composted soil when about 1 inch in height, and grown to sufficient size for etiolation. Figure 1 shows a typical flat of seedlings of a back-crossed progeny after etiolation.

INTERPRETATION OF EXPERIMENTAL RESULTS

In the interpretation of experimental results, probable errors and the χ^2 test for goodness of fit as outlined by Kirk and Immer (*6*) were employed.

Probable errors were taken from tables based on standard formulae; and values of χ^2 were taken from tables by Fisher (*3*).

FIELD RESULTS WITH LITTLE DUTCH × WHITE BURLEY

The data presented in Table 1 were brought together from the field notebooks of Doctor Johnson late in 1928. A small field planting of certain segregating progenies from cross 63 had been made by the writer during the summer of the same year. The results of this planting and the field results of the F_2 population planted in 1929 are included in these totals for the sake of completeness.[6]

Table 1 presents a summary of field segregation in F_2 and F_3 of Little Dutch × White Burley and in the back cross of the green F_1 to recessive White Burley. Inspection of the data listed reveals a rather poor fit on the basis of dihybrid segregation. There is in every case a deficiency of recessives considerably greater than ordinarily expected. Furthermore among the 20 progenies that make up the totals in Table 1, all but 3 were in turn recessive-deficient. In addition to the segregating F_3 families a number of true-breeding green and true-breeding white F_3 progenies were recovered by Johnson.

TABLE 1.—*Field counts on Little Dutch × White Burley (cross 63), 1928*

Designation	Progenies	Plants			Ratio	Deviation	Dev. / P. E.
		Green	White				
	Number	*Number*	*Number*	*Per cent*			
$63F_2$	4	4,371	167	3.7	15:1	−116.6±11.0	10.6
$63F_3$	8	1,732	92	5.0	15:1	−22.0± 7.0	3.1
$63F_3$	2	377	79	17.3	3:1	−35.0± 6.2	5.6
$63F_1$×WB	6	783	195	19.9	3:1	−49.9± 9.1	5.4

In general, two interpretations may be made of data of this character: (1) Inheritance may be of a simple Mendelian nature, and environmental factors may be considered as responsible for the elimination of recessives at some early stage; or (2) inheritance may be more complex, involving modifying factors and possibly linkage.

Attempts were made to check the recessive-deficient ratios observed by classifying the plants remaining in the seed beds at the conclusion of field planting to determine if selection based on a differential growth rate of green and white seedlings could be responsible for the deficiencies. In cases in which sufficient plants remained to yield a significant tally, ratios closely approximating those observed in field planting were obtained.

Examination of F_2 results and the data from the back-crossed F_1 generation disclosed no evidence of difference in reciprocal crosses, which rules out any hypothesis assuming differential pollen-tube growth.

[6] Typed copies of the data from which summary tables appearing in this manuscript were taken are available for inspection.

GREENHOUSE TESTS OF F_2 POPULATIONS

With the development of the greenhouse technic for the identification of the White Burley character in the seedling stage, the 1929 growing season was devoted to the production of suitable progenies for greenhouse analysis. A large planting of $63ZZZF_2$ was made from which some 250 green plants were selected at random and self-pollinated. Over 100 of these selections were also back crossed to pure-line White Burley to provide an additional check on their genetic constitution.

In addition F_1 plants from the cross 63ZZZ were self-pollinated and back crossed to White Burley.

In Table 2 are summarized the results of the greenhouse analysis of 12 F_2 families, 10 of which were obtained from self-pollinated plants of $63ZZZF_1$. Of the 12 populations investigated, 10 were recessive deficient on the basis of 15: 1 segregation, and in 4 of these cases the extent of the deviations was well beyond the limits of variability expected in random sampling. In the total of these populations involving more than 18,000 plants, the cumulative nature of recessive deficiencies in individual populations is apparent.

TABLE 2.—*Greenhouse counts on Little Dutch × White Burley (cross 63) and other green × white crosses; segregation in the F_2 generation*

Designation	Progenies	Plants			Deviation from 15:1 ratio	Dev. / P. E.
		Green	White			
	Number	*Number*	*Number*	*Per cent*		
$63F_2$	12	17,593	948	5.1	−210.8±22.2	9.5
$224F_2$	2	1,397	78	5.3	−14.2± 6.3	2.3
$232F_2$	4	1,680	85	4.8	−25.3± 6.8	3.7
$233F_2$	2	921	50	5.1	−10.7± 5.1	2.1

F_2 distributions from three other green by white crosses are also shown in Table 2. Although the magnitude of the deviations from expectation in these populations is not large, the deviations are all in the same direction, as in cross 63.

The results of a small field planting of these F_2s, for which data is not presented, further indicate that the factorial situation in the parents involved is similar to that in the Little Dutch × White Burley cross.

EVIDENCE FROM THE F_3 GENERATION

Greenhouse tests of 108 F_3 progenies from cross 63 resulted in the distribution with regard to segregating and nonsegregating families shown in Table 3.

TABLE 3.—*Greenhouse counts on 108 F_3 progenies from cross 63*

Ratio	All green	15 green: 1 white	3 green: 1 white
Observed	53	31	24
Expected, on basis of dihybrid segregation	50.4	28.8	28.8

χ^2=1.10215. P=0.75.

The agreement between observed and expected results in the distribution is excellent. This progeny test, involving the green segregates only, provides strong evidence of the dihybrid nature of the material. The F_3 tests are summarized in Table 4. Data for the greenhouse counts on the 53 nonsegregating F_3 families totaling over 24,600 plants are not included.

TABLE 4.—*Greenhouse counts on Little Dutch × White Burley (cross 63), the F_3 progenies segregating for one and for two factors*

Designation	Progenies	Plants			Ratio	Deviation	Dev. / P. E.
		Green	White				
	Number	*Number*	*Number*	*Per cent*			
$63F_3$	31	19,677	1,105	5.3	15:1	−193.9±23.5	8.3
	23	13,139	3,476	20.9	3:1	−677.8±37.6	18.0

Examination of the F_3 families segregating for two factors revealed that 26 of the total 31 progenies were recessive deficient. Among these individual progenies the extent of the recessive deficiencies was not great since only two progenies had deviations as large as three times their respective probable errors. Furthermore, in but two of the five families showing an excess of white segregates were the numbers sufficiently large to merit individual consideration. For the behavior of these two, 63–57 and 63–78, no good explanation can be advanced. It was noticed, however, in comparing the F_3 families with the results of back crosses of the parental F_2 that the back cross of the green F_2 plants in both of these cases gave populations deficient in recessives.

Additional evidence of the cumulative nature of the recessive deficiencies was supplied by the F_3 families segregating approximately 3 green : 1 white, also summarized in Table 4. Twenty-one of the 23 families tested were recessive deficient, and all progenies involving more than 600 plants showed deficiencies greater than three times their respective probable errors. On the basis of probable errors one might conclude that larger deficiencies occurred in the progenies segregating for a single factor than in those segregating for two factors. Actually, however, the percentage elimination of recessives based on the total number expected in the two cases is nearly the same—16.3 per cent recessive deficiency for the former, and 15.7 per cent for the latter.

In the back crosses of the parental F_2 selections summarized in Table 5 the percentage of recessive elimination was considerably less, namely, 7.1 per cent for progenies segregating 3 green : 1 white, and 2.2 per cent for those segregating in a 1:1 ratio. Although the inference to be drawn from this behavior is not wholly clear, it appears that the degree of recessive deficiency is influenced by the source of the material.

TABLE 5.—*Greenhouse counts on Little Dutch × White Burley (cross 63), segregation in back cross to White Burley of F_2 green selections which, on self pollination, yielded F_3 progenies segregating for one and for two factors*

Designation	Progenies	Plants			Ratio	Deviation	Dev. / P. E.
		Green	White				
	Number	*Number*	*Number*	*Per cent*			
$63F_2 \times WB$	18	5,885	1,779	23.2	3:1	−137.0±25.6	5.4
	15	2,708	2,588	48.9	1:1	−60.0±24.6	2.4

INVESTIGATION OF THE CAUSE OF THE RECESSIVE DEFICIENCY

In an attempt to discover the factors responsible for the recessive deficiencies observed, and to determine if possible the stage at which recessives were discriminated against, the following investigations were undertaken:

It was thought that certain phases of the greenhouse technic might have influenced the percentage of whites recovered, particularly the discarding of plants remaining in stock flats after transplanting. This failure to test all the plants raised in stock flats was appreciable only in the first few months of the greenhouse tests; thereafter effort was made to sow a sufficiently small quantity of seed of each progeny to enable practically all the plants grown to be tested. An analysis of the records for certain highly deficient progenies was made by separating the first and last half of the populations transplanted from stock flats for comparison with regard to the percentage of recessives in each half. These data are presented in Table 6 and show no evidence of a differential growth rate between dominant green and recessive white seedlings. It is, therefore, reasonable to assume that no great error was introduced in the few cases in which the surplus stock plants were not tested.

TABLE 6.—*Greenhouse counts on Little Dutch × White Burley (cross 63), showing effect of selection on percentage of whites recovered in successive transplantings of segregating progenies from stock flats*

Progeny No.	Transplanted to—	Plants			Deviation	Dev. / P. E.
		Green	White			
		Number	*Number*	*Per cent*		
F_3 PROGENIES RECESSIVE DEFICIENT FOR 3:1 RATIO						
63–22	Flats 1–8	767	194	20.2	−46.3± 9.1	5.1
	Flats 9–16	774	186	19.4	−54.0± 9.1	5.9
63–24	Flats 1–4	331	130	28.2	14.7± 6.3	2.3
	Flats 5–8	391	89	18.5	−31.0± 6.4	4.8
63–33	Flats 1–3	730	169	18.8	−55.8± 8.8	6.3
	Flats 4–6	691	168	19.6	−46.8± 8.6	5.4
63–38	Flats 1–4	966	275	22.2	−35.3±10.3	3.4
	Flats 5–9	1,150	341	22.9	−31.8±11.3	2.8
F_3 PROGENIES DEFICIENT FOR 15:1 RATIO						
$B63F_2$	Flats 1–4	905	41	4.3	−18.1±5.0	3.6
	Flats 5–8	924	40	4.1	−20.3±5.1	4.0
$H63F_2$	Flats 1–3	797	28	3.4	−23.6±4.7	5.0
	Flats 4–6	904	45	4.7	−14.3±5.0	2.9
$J63F_2$	Flats 1–3	901	40	4.3	−18.8±5.0	3.8
	Flats 4–5	593	24	3.9	−14.6±4.1	3.6
$K63F_2$	Flats 1–3	913	42	4.4	−17.7±5.1	3.5
	Flats 4–6	907	42	4.4	−17.3±5.0	3.5

Attention was next devoted to a series of controlled experiments in which counted numbers of seed were used and a special effort was made to keep the moisture content of the soil at the optimum for germination. Since, in transplanting, only seedlings that had attained a certain size were removed from the stock flats at a given time, record was kept of successive transplantings to give further check on the possibility of a differential growth rate between green and white seedlings.

The results of such an experiment are shown in Table 7. One thousand seeds of each of six progenies were sown on greenhouse flats, and all plants were tested. The data show no evidence of differential growth rate between green and white seedlings.

TABLE 7.—*Greenhouse counts on F_2, F_3, and back crossed F_1 of Little Dutch × White Burley (cross 63)*

[Analysis of successive transplantings from 1,000 seed sown on stock flats to show effect of selection in transplanting on percentage of whites recovered from segregating progenies]

Progeny No.	Transplanting No.	Plants			Deviation from ratio indicated	Dev. / P. E.
		Green	White			
		Number	*Number*	*Per cent*		
C63F_2	1	249	21	7.8	4.1 from 15:1	1.5
	2	253	23	8.3	5.8 from 15:1	2.1
	3	95	6	5.9	−.3 from 15:1	.2
Total		597	50	7.7	9.6±4.2 from 15:1	2.3
C63F_1 × White Burley	1	160	47	22.7	−4.8 from 3:1	1.1
	2	27	5	15.6	−3.0 from 3:1	1.7
Total		187	52	21.8	−7.8±4.5 from 3:1	1.7
63–24 (F_2)	1	186	53	22.2	−6.8 from 3:1	1.5
	2	147	39	21.0	−7.5 from 3:1	1.9
	3	148	61	29.2	8.8 from 3:1	2.1
	4	89	29	24.6	−.5 from 3:1	.1
Total		570	182	24.2	−6.0±6.7 from 3:1	.9
63–26	1	191	62	24.5	−.3 from 3:1	.1
	2	161	59	26.8	4.0 from 3:1	.9
	3	43	14	24.6	−.3 from 3:1	.1
Total		395	135	25.5	2.5±6.7 from 3:1	.4
63–28	1	262	20	7.1	2.4 from 15:1	.9
	2	229	19	7.7	3.5 from 15:1	1.8
	3	141	9	6.0	−.4 from 15:1	.2
	4	55	8	12.7	4.1 from 15:1	3.1
Total		687	56	7.5	9.6±4.5 from 15:1	2.1
63–34	1	175	18	9.3	5.9 from 15:1	2.6
	2	269	29	9.7	10.4 from 15:1	3.7
	3	235	6	2.5	−9.1 from 15:1	3.6
	4	39	5	11.4	2.3 from 15:1	2.1
Total		718	58	7.5	9.5±4.6 from 15:1	2.1

In the second part of this experiment, which was intended as a check on the soil tests, seed was germinated on moist filter paper in Petri dishes to give each seed the maximum chance to produce a plant. After 10 days, the very young seedlings were transferred to soil by removing the filter paper, placing it on a leveled flat, and covering it with a thin layer of finely sifted soil. Since in the second week following this transfer many seedlings died, this test can not be considered in the light of a check on the same progenies germinated

in soil. Nevertheless, the data shown in Table 8 are of interest because of their close agreement with theoretical expectation for the ratios involved. In view of the rigorous treatment to which the plants were subjected, such a result indicates that recessive elimination must be sought for during germination or at some earlier stage in the life cycle.

TABLE 8.—*Greenhouse counts on Little Dutch × White Burley (cross 63), when 500 seed of each segregating progeny were germinated in Petri dishes and transferred to soil*

Progeny No.	Plants			Deviation from ratio indicated	Dev./P.E.
	Green	White			
	Number	*Number*	*Per cent*		
C63F$_1$×White Burley	86	28	24.6	−0.5±3.1 from 3:1	0.2
63-22	73	24	24.7	−.3±2.9 from 3:1	.1
63-24	78	25	24.3	−.8±3.0 from 3:1	.3
63-26	73	27	27.0	2.0±2.9 from 3:1	.7
Total	310	104	25.1	.5±5.9 from 3:1	.1
63-28	56	4	6.7	.3±1.3 from 15:1	.2
63-34	263	14	5.1	−3.3±2.7 from 15:1	1.2
Total	319	18	5.3	−3.1±3.0 from 15:1	1.0

THE EFFECT OF THICKNESS OF SOWING

It seemed possible that the thickness of sowing might be one of the factors affecting the percentage of recessives germinating in segregating populations. In another experiment, therefore, seed of seven back-crossed progenies was sown at three different rates in partitioned greenhouse flats. Germination tests on additional seed of these progenies were run in Petri dishes, and the resulting seedlings were transferred to soil by "flooding off" with a fine-tipped wash bottle.

The results of the different rates of sowing in soil are presented in Table 9. It will be noted that the total number of plants yielded by the sowing on whole flats checks very closely with the total for the sowing on one-eighth flats, whereas from comparable seed sown on one-quarter flats only about half as many plants were obtained. Since the soil in these tests was thoroughly mixed before sterilization, this inconsistency is probably due to insufficient moisture during the crucial stages of germination, in spite of the care taken to prevent such an occurrence.

TABLE 9.—*Greenhouse counts on Little Dutch × White Burley (cross 63), showing effect of thickness of sowing on the green : white ratio obtained with 3,500 seed of back-crossed F$_2$ selections sown on different areas*

Designation	Area sown	Plants			Deviation from 1:1 ratio	Dev./P.E.
		Green	White			
	Square inches	*Number*	*Number*	*Per cent*		
63F$_2$×WB	300	638	601	48.5	−18.5±11.9	1.6
	75	314	297	48.6	−8.5± 8.3	1.0
	37.5	606	637	51.2	15.5±11.9	1.3

That the percentages of white segregates were not greatly affected, however, is shown by a comparison of the totals of each group. The irregular behavior of the individual progenies within the three groups make any statistical interpretation a difficult procedure. There was, however, a slight increase in the percentages of recessives obtained as the thickness of sowing increased.

The results of the Petri dish transfers are given in Table 10. With individual back crosses the full quota of recessives has been recovered in four cases out of seven, which is a normal expectation. Although seedling mortality in these transfers was again rather high, the results, in comparison with those of the same progenies germinated in soil, indicate that the actual elimination of recessives takes place during germination, although the effect of the recessive genotype may be exerted to some extent earlier in the ontogeny of the seed.

TABLE 10.—*Greenhouse counts on Little Dutch × White Burley (cross 63), when 500 seed of each back-crossed F_2 selection were germinated in Petri dishes and then transferred to soil*

Progeny No.	Germination	Plants surviving transfer	Plants			Deviation from 1:1 ratio	Dev. P. E.
			Green	White			
	Per cent	*Number*	*Number*	*Number*	*Per cent*		
63-22×White Burley	37	100	53	47	47.0	−3 ±3.4	0.9
63-24×White Burley	57	160	80	80	50.0	0 ±	
63-25×White Burley	76	132	68	64	48.5	−2 ±3.9	.5
63-26×White Burley	85	188	93	95	50.5	1 ±4.6	.2
63-27×White Burley	85	360	179	181	50.3	1 ±6.4	.2
63-47×White Burley	86	406	201	205	50.5	2 ±6.8	.3
63-61×White Burley	73	227	118	109	48.0	−4.5 ±5.1	.9
Total	71.3	1,573	792	781	49.7	−5.5±13.4	.4

EFFECT OF WEIGHT OF SEED

To test for the effect of the weight of the seed, seed of each of seven F_3 families was separated into two weight classes, heavy and light, and counted numbers of each were sown on soil and in Petri dishes. After germination the seedlings in Petri dishes were transplanted individually to soil and grown to sufficient size for etiolation. Final counts on these transfers listed in Tables 11 and 12 show a small difference in the percentages of recessives recovered from heavy and light seed sown in soil, and a slightly smaller difference between these two classes with the seed germinated in Petri dishes. Closer examination of these tables reveals that two of the individual progenies, namely, 63–24 and 63–26, yielded a slight excess of recessives in three of the four tests. They were recessive deficient only in the test of light seed germinated in Petri dishes.

Within the limits of this experiment the behavior of these progenies is characterized by a degree of consistency which, in comparison with earlier tests, seems too great to be attributed to chance alone.

By consulting the deviations observed with the Petri dish transfers in Table 12, it is found that the recessive deficiencies are distributed at random with regard to the germination percentages of the progenies. This is to be expected since in these tests comparatively weak seeds have a better chance to germinate. However, a similar comparison of the germination percentages in this table with the deviations observed

in the soil tests shown in Table 11 reveals that in the light-seed class the two positive deviations were produced by 63–24 and 63–26, two progenies with germination percentages of 86 and 91 per cent, respectively. Also, 63–61, the only other progeny whose light seed tested over 80 per cent in Petri dishes, has a negligible deviation in the soil test of −0.5.

TABLE 11.—*Greenhouse counts on Little Dutch × White Burley (cross 63), showing the effect of weight of seed on the green : white ratio obtained with 500 heavy and 500 light seed of segregating F_3 progenies sown on one greenhouse flat*

Light or heavy seed	Progeny No.	Plants			Deviation from 3 : 1 ratio	Dev. / P. E.
		Green	White			
		Number	*Number*	*Per cent*		
Light	63–22	112	32	22.2	−4.0± 3.5	1.1
	63–24	178	61	25.5	1.3± 4.5	.3
	63–25	139	34	19.7	−9.3± 3.8	2.4
	63–26	164	57	25.8	1.8± 4.3	.4
	63–27	179	51	22.2	−6.5± 4.4	1.5
	63–47	72	11	13.3	−9.8± 2.7	3.6
	63–61	230	76	24.8	−.5± 5.1	.1
	Total	1,074	322	23.1	−27.0±10.9	2.5
Heavy	63–22	132	31	19.0	−9.8± 3.7	2.6
	63–24	241	88	26.7	5.8± 5.3	1.1
	63–25	245	69	22.0	−9.5± 5.2	1.8
	63–26	186	81	30.3	14.3± 4.8	3.0
	63–27	184	54	22.7	−5.5± 4.5	1.2
	63–47	144	40	21.7	−6.0± 4.0	1.5
	63–61	223	71	24.1	−2.5± 5.0	.5
	Total	1,355	434	24.3	−13.3±12.4	1.1

TABLE 12.—*Greenhouse counts on Little Dutch × White Burley (cross 63), showing the effect of weight of seed on the green : white ratio obtained with 300 heavy and 300 light seed of segregating progenies germinated in Petri dishes and then transferred to soil*

Progeny No.	Light or heavy seed	Average germination	Seedlings transferred	Seedlings surviving	Plants			Deviation from 3 : 1 ratio	Dev. / P. E.
					Green	White			
		Per cent	*Number*	*Number*	*Number*	*Number*	*Per cent*		
63–22	Light	65	197	188	140	48	25.5	1.0± 4.0	0.3
63–24		86	258	252	192	60	23.8	−3.0± 4.6	.7
63–25		77	232	219	158	61	27.9	6.3± 4.3	1.5
63–26		91	274	254	194	60	23.6	−3.5± 4.7	.7
63–27		58	174	169	130	39	23.1	−3.3± 3.8	.9
63–47		54	164	160	117	43	26.9	3.0± 3.7	.8
63–61		95	287	276	216	60	21.7	−9.0± 4.9	1.8
Total		-------	1,586	1,518	1,147	371	24.4	−8.5±11.4	.7
63–22	Heavy	97	291	291	224	67	23.0	−5.8± 5.0	1.2
63–24		98	305	[a] 305	228	77	25.2	.8± 5.1	.2
63–25		97	290	259	198	61	23.6	−3.8± 4.7	.8
63–26		99	297	297	216	81	27.3	6.8± 5.0	1.4
63–27		97	290	269	211	58	21.6	−9.3± 4.8	1.9
63–47		97	292	265	196	69	26.0	2.8± 4.8	.6
63–61		97	292	279	206	73	26.2	3.3± 4.9	.7
Total		-------	2,057	1,965	1,479	486	24.7	−5.3±13.0	.4

[a] 310 seed instead of 300 used in this sample.

It is evident that progenies with the lowest germination percentages in Petri dishes are most deficient in recessives. The effect is somewhat less pronounced with the heavy seeds, but here 63–24 and 63–26 again produce a small excess of recessives. This behavior is significant since

it indicates that the different progenies contain variable percentages of weak seed which germinate in Petri dishes but do not germinate so successfully in soil. Evidently it is among the weaker seeds that recessive elimination occurs.

There remain to be presented germination experiments with mixtures of true-breeding green and white families. In the first of these seed of Little Dutch, the green parent in cross 63, was mixed with seed of 63–31, a White Burley type segregate from cross 63, and the mixture sown on soil in a greenhouse flat. After the seedlings were transplanted and etiolated in the usual manner, the percentage of white plants resulting from the mixed sowing was compared with that expected, using separate soil-germination tests of the two component lines as a basis for calculation. As is apparent from the data in Table 13 the relative germination percentage of the recessive White Burley type seed was markedly decreased by the conditions of mixed sowing.

TABLE 13.—*Greenhouse counts on a mixture of pure-line green and pure-line white seed sown in soil*

Designation	Seed sown	Transplanted to flat No.	Plants			Percentage green on basis of total plants obtained	Percentage white on basis of total plants obtained	Deviation	Dev./P. E.
			Green	White					
	Number		*Number*	*Number*	*Per cent*			*Per cent*	
63-31 (white)	1,000		0	856			59.8		
Little Dutch	1,000		576	0		40.2			
63-31 + Little Dutch.	1,000+ 1,000	1	144	165	53.4				
		2	156	109	41.1				
		3	88	153	63.5				
		4	111	169	60.4				
		5	32	21	39.6				
Total			531	617	53.7	46.3	53.7	[a]−6.1±0.870	7.0

[a] Expectation calculated on basis of respective germination of green and white checks sown separately.

To test this point further, green and white stocks were made up from seed of true-breeding F_3 progenies having germinative capacities more nearly equal, with the object of balancing out any physiological weakness present in seed of a single family. The composition of these stocks and their respective germination percentages in Petri dishes are given in Table 14.

TABLE 14.—*Composition of green and white stocks and germination of constituent progenies in Petri dishes*

Designation of stock	Constituent progenies	Germination in 10 days	Average germination
		Per cent	*Per cent*
Green A	63-30	93	92
	63-32	92	
	63-36	90	
White A	63-31	97	95
	63-225	92	
	63-20	96	
White B	A232-21	85	89
	A232-22	95	
	A232-23	88	

Seed of the three stocks was sown separately and in mixtures at different rates on greenhouse flats. The results of this sowing, shown in Table 15, are somewhat contradictory. The green A stock yielded a significantly greater number of plants from 600 seeds sown on one-quarter flat than from an equal number of seeds sown on a whole flat, while the reverse is true of the white A and white B stocks. The low actual yield of plants in this particular test suggests the operation of some uncontrolled environmental factor during germination, an opinion supported by repetition of the check sowings at different rates as shown in the lower part of Table 16. Additional evidence that crowded sowing induces a higher yield of plants is also presented in Table 16. In this case the constituent progenies of the three stocks were tested separately and showed consistent increases in the number of plants obtained at the thicker sowing. A comparison of these data with those of the Petri dish germination tests shown in Table 14 reveals a congruity of behavior on the part of the stocks and their constituent progenies under the two conditions. There is no evidence in these tests that crowded sowing in soil results in discrimination against the recessive genotype.

TABLE 15.—*Greenhouse counts on mixtures of green and white stocks sown on different areas in soil*

Stock	Seed sown	Area sown	Plants obtained	Plants		
				Green	White	
	Number		*Number*	*Number*	*Number*	*Per cent*
Green-A	600	1 flat	145			
White-A	600	do	273			
White-B	600	do	236			
Green-A	600	¼ flat	242			
White-A	600	do	146			
White-B	600	do	128			
Green-A+white-A	600+600	1 flat		260	232	47.2
Green-A+white-B	600+600	do		117	172	59.5
Green-A+white-A	600+600	¼ flat		161	161	50.0
Green-A+white-B	600+600	do		145	142	49.5

TABLE 16.—*Greenhouse counts on constituent progenies of green and white stocks from 600 seeds sown separately in soil*

Stock	Constituent progenies	Area sown	Plants recovered	Total plants per stock
			Number	
Green-A	63-30	1 flat	308	1,033
	63-32		408	
	63-36		317	
White-A	63-31		469	1,172
	63-225		283	
	63-20		420	
White-B	A232-21		344	1,153
	A232-22		404	
	A232-23		405	
Green-A	63-30	¼ flat	436	1,461
	63-32		514	
	63-36		511	
White-A	63-31		557	1,508
	63-225		472	
	63-20		479	
White-B	A232-21		430	1,392
	A232-22		493	
	A232-23		469	
Green-A		½ flat	352	
White-A			388	
White-B			411	
Green-A		⅛ flat	458	
White-A			516	
White-B			513	

DISCUSSION

Considering the whole of the data presented on segregation in F_2, F_3, and back crosses of F_1, and F_2 of Little Dutch × White Burley as well as the F_2 results of other green by white crosses, the genetic behavior of the White Burley character is best explained on the basis of duplicate factors which may be designated G_1 g_1 G_2 g_2. Since F_2 segregation in several other green by white crosses closely parallels that observed in Little Dutch × White Burley it is probable that green and White Burley varieties commonly differ in these same two factor pairs.

On the basis of two independent duplicate Mendelian factors one would expect to recover in F_2 6.25 per cent of the recessive white genotype g_1 g_1 g_2 g_2. Normally the deviations from this theoretical percentage should be equally distributed as to direction. That this expectation is not realized with progenies of Little Dutch × White Burley is evident from a consideration of almost any table of field or greenhouse results presented. The nature of the recessive deficiencies is clearly shown in Table 2, where segregation in 12 F_2 families grown in the greenhouse is recorded. It is equally apparent in Table 4, where small individual deficiencies lead to a total deficiency of over eight times the probable error.

That the recessive deficiencies are not caused by auxiliary genetic factors operating to modify the normal dihybrid ratio seems fairly clear. The similarity of reciprocal crosses and back crosses, and the contradictory behavior of certain F_2 and F_3 progenies in the field and in the greenhouse discourage attempts to account for the deficiencies on any such basis. Likewise, errors in classification and effect of selection in transplanting may be ruled out.

Since no deficiency of recessives was observed in the Petri dish transfers of several experiments, the actual elimination of recessives seems to occur during germination in soil. In this connection attention is again called to the physiological nature of the seed used in these experiments. F_3 progenies have been shown to contain variable amounts of seed which will germinate in Petri dishes but not in soil. Furthermore, seed of high germination percentage in the Petri dish experiments yielded recessives in expected numbers, whereas seed of low germination percentage did not. Differential zygotic viability, therefore, between dominant green and recessive white seems to be the most logical explanation of the recessive deficient ratios observed. On this basis one would expect a progressive increase in the degree of recessive deficiency between sowings in Petri dishes, in greenhouse flats, and in exposed seed beds as the control of optimum conditions for germination is relaxed. Such an increase has been observed.

The work of Allard (*1*) on the aurea character in *Nicotiana rustica* is interesting in this connection. Segregation in the F_2 generation of a green by aurea cross involving a total of 25,000 plants yielded 6,079 aureas, or 24.31 per cent. This total is apparently a summation of 13 separate F_2 plantings, 11 of which showed small recessive deficiencies. The minus deviation from expected numbers in this total, however, is about 3.7 times its probable error. The total of the segregating F_3 families and of the F_1 back crossed to white-stemmed aurea also show small deficiencies in the recessive class. Appearance of chlorophyll-

deficient recessives in less than expected numbers, therefore, seems to be characteristic of this species of Nicotiana as well.

Similar phenomena have been reported by other investigators, particularly with maize, where the existence of many recessive abnormalities has been established. Lindstrom (7) studied a number of recessive chlorophyll modifications affecting the mature corn plant. In populations segregating for these characters slight recessive deficiencies were regularly encountered.

In tobacco it is probable that the degree of recessive deficiency is materially influenced by environmental conditions during seed development. It is conceivable that back-crossed seed involving the maturation of only a few capsules per plant would be physiologically stronger than self-fertilized seed from a plant maturing many capsules. Certain of the results obtained with self-fertilized and back-crossed seed of the same progenies might be explained on this basis.

SUMMARY

The inheritance of the chlorophyll-deficient White Burley character in crosses of White Burley with normal green tobaccos has been studied in field plantings, in greenhouse plantings, and in transfers to soil of seed germinated in Petri dishes. A special technic for the identification of this character in greenhouse seedlings has been developed and was employed in the classification of segregating progenies.

Data from the F_1, F_2, and F_3 generations and from back crosses of F_1 and F_2 show that duplicate genes designated G_1 and G_2 are involved in the production of the normal green color of the plant. Their recessive allelomorphs g_1 and g_2 are both essential to the White Burley genotype. F_1 plants are fully green, and segregation in F_2 is accordingly 15 green: 1 white. In F_3 true-breeding green and true-breeding white progenies were recovered as well as families segregating for one and for two factors.

Large recessive deficiencies in field plantings, and smaller but equally distinct recessive deficiencies in greenhouse plantings were observed in all segregating generations. Evidence from successive greenhouse transplantings, and data from experiments involving counted numbers of seed show that selection from field seed beds and from greenhouse stock flats is not responsible for these deficiencies.

Experiments with counted numbers of seeds show that crowded sowing does not cause the elimination of recessives since frequently increases in the percentage of germination paralleled by slight increases in percentage of recessives recovered were obtained with thickly sown seed.

Evidence that environmental and not genetic factors are concerned in the nonappearance of recessives in expected numbers is fourfold: (1) No significant differences are observed in the F_2 of reciprocal crosses and in reciprocal back crosses of the green F_1 to White Burley; (2) the differences between field and greenhouse results are most logically explained on the basis of nongenetic influence; (3) certain F_3 progenies produce an excess of recessives in some greenhouse experiments and a deficiency in others; and (4) in some Petri dish transfers normal expectation for recessives is realized.

Experiments with heavy and with light seed germinated in soil and in Petri dishes demonstrate that segregating progenies normally contain variable amounts of weak seed which will germinate in Petri dishes but not in soil. Elimination of recessives in these experiments tends to decrease with an increase in relative viability of the light seed.

A differential viability between dominant green and recessive white genotypes is therefore apparently responsible for the recessive deficiencies observed.

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AN ADDITIONAL PAIR OF FACTORS AFFECTING ANTHOCYANIN PIGMENT IN MAIZE[1]

By MERLE T. JENKINS

Associate Agronomist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

This paper reports a new gene affecting the production of anthocyanin pigment in maize (*Zea mays* L.). This gene is so similar in its action, both in aleurone and in plant color, to the *Aa* factor pair reported by Emerson[2] in 1918 that it has been called A_2a_2. It is suggested that the *Aa* factor pair isolated by Emerson be designated A_1a_1.

MATERIAL

The A_2a_2 factor pair was obtained from Iodent corn, a strain of Reid Yellow dent developed at the Iowa Agricultural Experiment Station.[3] The original progeny heterozygous for A_2a_2 also was heterozygous for the chlorophyll defect, iojap. In fact, the material was being used for a study of this chlorophyll defect, and the presence of a_2 was not suspected until peculiar ratios of aleurone colors were obtained. Many of the data presented in this paper have come from the F_1, F_2, and later generations of an individual cross between the a_2 stock and an A_1 tester for aleurone color which was homozygous for brown plant color. The genetic composition of this cross was as follows:

$$a_1a_1\ A_2A_2\ CC\ RR\ BB\ Pl\ Pl\ p\ p \times A_1A_1\ A_2a_2\ cc\ rr\ bb\ pl\ pl\ P^{wr}\ P^{wr}$$

This cross will be referred to throughout as the original F_1 cross.

INTERACTION OF A_2 WITH ALEURONE GENES A_1, C, AND R

Aleurone color in maize is dependent upon the presence of the dominant allelomorphs of the three complementary pairs of genes A_1a_1, *Cc*, and *Rr* and the duplex recessive condition of the inhibitor, *Ii*. The interactions of these genes are so familiar to every student of maize genetics that they need not be reviewed here. In its effect on aleurone color the new gene, A_2, is complementary to the three complementary genes mentioned above.

Fifty-five of the plants of the original F_1 cross were self-pollinated and yielded F_2 progenies. Twenty-four of these progenies segregated for aleurone color in the proportion of 27 colored to 37 colorless, and

[1] Received for publication Nov. 2, 1931; issued May, 1932. The data on which this paper is based come from the corn-improvement project conducted cooperatively by the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Farm Crops Section of the Iowa Agricultural Experiment Station.

[2] EMERSON, R. A. A FIFTH PAIR OF FACTORS, Aa, FOR ALEURONE COLOR IN MAIZE, AND ITS RELATION TO THE Cc AND Rr PAIRS. N. Y. Cornell Agr. Expt. Sta. Mem. 16, p. 227–289, illus. 1918.

[3] The writer is informed by G. W. Beadle, of the New York State College of Agriculture, Ithaca, N. Y., that he isolated a_2 from some Argentine flint corn collected by F. D. Richey and R. A. Emerson at Casilda, Argentina.

the remaining 31 F_2 progenies segregated for aleurone color in proportions approximating 81 colored to 175 colorless. Equal numbers of each kind of segregation were expected. The deviation of 3.5 is 1.4 times its probable error. A summary of the data from these 55 progenies is recorded in Table 1.

TABLE 1.—*Summary of data on 55 F_2 progenies*

Ratio	Number of progenies	Colored		Colorless		Dev. / P. E.
		Observed	Expected	Observed	Expected	
27 : 37	24	4,622	4,693	6,501	6,430	0.67
81 : 175	31	4,278	4,539	10,067	9,806	2.18

Six of the F_2 progenies showing aleurone color ratios of 81:175 were grown in the field, and a large number of the plants from colored seeds were self-pollinated. A summary of the aleurone color segregations of the 123 F_3 progenies obtained is recorded in Table 2.

TABLE 2.—*Data on F_3 aleurone color segregations obtained by selfing F_2 plants produced by colored seeds from 81 : 175 ratios*

Aleurone ratio	Number of F_3 progenies		Deviation
	Observed	Expected	
All colored	0	1.5	−1.5
3 colored to 1 colorless	11	12.2	−1.2
9 colored to 7 colorless	37	36.5	+.5
27 colored to 37 colorless	50	48.6	+1.4
81 colored to 175 colorless	25	24.3	+.7
Total	123	123.1	−.1

$\chi^2 = 1.69$. $P = 0.8-$.

A number of the 9:7 F_3 progenies were grown in the field and tested to determine which of the aleurone factors were heterozygous. Among those grown there were some heterozygous for A_2a_2 and each of the factor pairs A_1a_1, *Cc*, and *Rr*. Plants from the colored seeds in these progenies were self-pollinated. They yielded F_4 progenies, of which some were homozygous colored, some segregated in ratios of 3 colored to 1 colorless, and some segregated for 9 colored to 7 colorless. A summary of the number of progenies showing each kind of segregation is recorded in Table 3.

TABLE 3.—*Summary of F_4 aleurone color segregations obtained by selfing F_3 plants produced by the colored seeds from the dihybrid ratios involving A_2a_2 and each of the other complementary genes for aleurone color*

Genes heterozygous	Number of progenies giving aleurone ratios indicated						Total
	All colored		3 colored to 1 colorless		9 colored to 7 colorless		
	Observed	Expected	Observed	Expected	Observed	Expected	
A_2a_2 and A_1a_1	3	5.6	27	22.2	20	22.2	50
A_2a_2 and *Cc*	4	1.8	5	7.1	7	7.1	16
A_2a_2 and *Rr*	0	2.8	16	11.1	9	11.1	25

A summary of the dihybrid segregations among these F_4 progenies which involved A_2a_2 and each of the other complementary factors for aleurone color is recorded in Table 4.

TABLE 4.—*Summary of F_4 dihybrid segregations involving A_2a_2 and each of the other complementary genes for aleurone color*

Genes heterozygous	Number of progenies	Observed		Expected		Dev. / P. E.
		Colored	Colorless	Colored	Colorless	
A_2a_2 and A_1a_1	20	4,339	3,481	4,399	3,421	2.0
A_2a_2 and Cc	7	1,379	1,061	1,373	1,067	.4
A_2a_2 and Rr	9	1,618	1,290	1,636	1,272	1.0

Frequency distributions of the aleurone color segregations obtained in the F_2, F_3, and F_4 progenies, summarized in Tables 2 and 3,

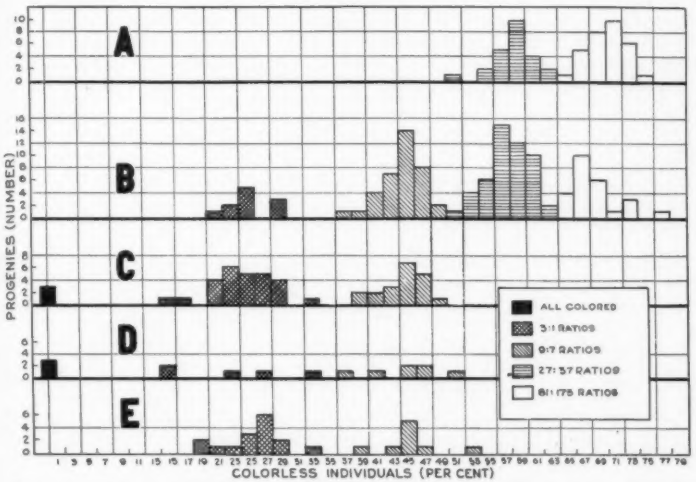

FIGURE 1.—Frequency distributions of aleurone color: A, F_2 progenies; B, F_3 progenies from plants produced by colored seeds of 81:175 progenies from A; C, D, and E, F_4 progenies from plants produced by the colored seeds of 9:7 progenies from B. C is from the 9:7 progenies heterozygous for A_1a_1 and A_2a_2, D from those heterozygous for A_2a_2 and Cc, and E from those heterozygous for A_2a_2 and Rr

are shown in Figure 1. It will be seen that there was some overlapping of the different kinds of ratios. This overlapping, however, does not appear to be of sufficient extent to discredit the progeny classifications presented in Tables 2 and 3.

The data presented in this section indicate that A_2 is complementary to A_1, C, and R in the production of aleurone color. They also indicate that A_2 is inherited independently of these three genes. More complete proof, however, that A_2 is not linked in inheritance with A_1, C, or R is presented in the section on linkage.

INTERACTION OF A_2 WITH PLANT COLOR GENES A_1, B, AND Pl

The interaction of the A_1, *B*, and *Pl* factors has been reported by Emerson (1921).[4] Gene A_2 is complementary to A_1 in its action upon plant color as well as in its action upon aleurone color. The F_1 plants of the original previously mentioned cross, which were heterozygous for A_2a_2, were of the constitution a_1A_2 *C R B Pl.* A_1a_2 *c r b pl.* The F_2 progenies contained seedlings with red and with green stems in the ratio of 9 red to 7 green. Six F_2 progenies were grown to maturity. The F_2 plant color segregations obtained and those expected on the assumption that A_2 is complementary to A_1 are indicated in Table 5.

TABLE 5.—*F_2 plant color segregations*

Genotypes		Phenotypes	Observed	Expected	Deviation
Composition	Number				
A_1 A_2 *B Pl*	81	Purple	306	306	0
A_1 A_2 *B pl*	27	Sun red	100	102	−2
A_1 A_2 *b Pl*	27	Dilute purple	93	102	−9
A_1 A_2 *b pl*	9	Dilute sun red	26	34	−8
A_1 a_2 *B Pl*	27				
a_1 A_2 *B Pl*	27	Brown	255	238	+17
a_1 a_2 *B Pl*	9				
A_1 a_2 *b Pl*	9				
a_1 A_2 *b Pl*	9				
A_1 a_2 *B pl*	9				
a_1 A_2 *B pl*	9				
A_1 a_2 *b pl*	3	Green	187	185	+2
a_1 A_2 *b pl*	3				
a_1 a_2 *B pl*	3				
a_1 a_2 *b Pl*	3				
a_1 a_2 *b pl*	1				
Total	256		967	967	0

$\chi^2=3.98$. $P=0.5+$.

The greatest differences between the observed and expected numbers occur in the dilute purple, dilute sun red, and brown classes. The differences suggest a loose linkage, in the coupling phase, between A_2 and *B*.

F_3 progenies were grown from a number of self-pollinated F_2 plants. A summary of the F_3 plant color segregations in the progenies from some of the F_2 plants heterozygous for A_2a_2 is recorded in Table 6.

All of these segregations are in agreement with the hypothesis that A_2a_2 is complementary to A_1a_1 in its action on plant color.

TABLE 6.—*Data on the plant color segregations in F_3 progenies from F_2 plants which were heterozygous for A_2a_2*

Group	Genetic composition	Pedigree	Purple	Sun red	Dilute purple	Dilute sun red	Brown	Green	Total
1 (A_1 A_1)	A_1A_1 A_2a_2 *BB Pl Pl*	5710	42				19		61
	Expected		46				15		61
	A_1A_1 A_2a_2 *BB Pl pl*	5706	40	12			9	2	63
	Expected		36	12			12	4	64
	A_1A_1 A_2a_2 *Bb Pl Pl*	5714	41		12		10	5	68
	Expected		38		13		13	4	68
	A_1A_1 A_2a_2 *bb Pl pl*	5722&8			65	18		36	119
	Expected				67	22		30	119
2 (A_1 a_1)	A_1a_1 A_2a_2 *BB Pl pl*	5690&4	49	20			29	17	115
	Expected		49	16			38	13	116
	A_1a_1 A_2a_2 *Bb Pl Pl*	5696	22		9		22	2	55
	Expected		23		8		18	6	55
	A_1a_1 A_2a_2 *Bb pl pl*	5700		25		4		26	55
	Expected			23		8		24	55
	A_1a_1 A_2a_2 *Bb pl pl*	5702		21		10		24	55
	Expected			23		8		24	55

[4] EMERSON, R. A. THE GENETIC RELATIONS OF PLANT COLORS IN MAIZE. N. Y. Cornell Agr. Expt. Sta. Mem. 39, 1–156 pp., illus. 1921.

INTERACTION OF A_2 WITH PERICARP GENE P^{wr}

The interaction of factors in the production of the red series of pericarp colors has been shown by Emerson [5] and by Anderson and Emerson [6] to be as follows:

A_1 P=red.
a_1 P=brown.
A_1 p=colorless.
a_1 p=colorless.

The original F_1 cross was heterozygous for P^{wr} (white pericarp, red cob) and p. The presence of brown plants with red cobs in the F_2 progenies segregating for A_2a_2 indicated that recessive a_2 must not affect pericarp and cob color in the same manner as recessive a_1. The F_2 distributions obtained, however, were not in agreement with those expected on the assumption that A_2a_2 had no influence whatever upon cob color. An excess of brown plants with brown cobs was obtained which could not be accounted for. During the past season several progenies were grown which were segregating for A_2a_2 and P^{wr} p and were homozygous for A_1A_1. Only red and white cobs were expected in these progenies. The unexpected occurrence of brown plants with brown cobs led to a more careful examination of the colored portions of these cobs. It developed that the color in these exceptional cobs was due to brown color located in the horny basal portion of the lower glumes and that the thin upper glumes or chaff were white.

In the mature pistillate spikelet of maize the lower (empty) glumes are thick and horny one-half to three-quarters of the distance from base to tip. They usually have thin membranous margins varying greatly in width. The upper glumes (lemmas and paleas) are thin and membranous.

The color produced by the pericarp gene P^{wr} appears to be confined to the thin upper glumes and to the thin margins of the lower glumes. The color of these parts will be referred to as upper glume or chaff color.

The color of the horny portions of the lower glumes (which will be termed lower glume color) is associated with plant color. In the cultures used in this study the basic color of the lower glumes on the ears of dilute sun red plants was light yellow or straw color. The color of these glumes was modified by the plant color factors and was correlated with plant color as follows:

Plant color	Lower glume color
Purple	purple.
Sun red	straw.
Dilute purple	straw, occasionally showing considerable purple.
Dilute sun red	straw.
Brown	brown.
Green	straw.

These colors doubtless vary somewhat in intensity in different cultures. Purple and dilute purple plants from different cultures show considerable variation in the amount of purple in the lower glumes, and brown plants in the amount of brown color. There also is much difference in the size of both upper and lower glumes in different cul-

[5] EMERSON, R. A. Op. cit. (See footnote 4.)
[6] ANDERSON, E. G., and EMERSON, R. A. PERICARP STUDIES IN MAIZE. I. THE INHERITANCE OF PERICARP COLORS. Genetics 8: [466]-476. 1923.

tures and consequently in their relative importance in determining the superficial color of the cob.

The basic colors of the upper glumes in the material studied were red and white. These colors were modified considerably on purple, dilute purple, and brown plants by the extension of purple and brown pigment into them. In the material used no difficulty was experienced, however, in determining whether their basic color was red or white, except in a few of the purple plants.

Gene a_2, unlike gene a_1, has no influence upon the upper glume color produced by the pericarp gene P. It influences lower glume color, however, in a manner exactly similar to a_1. On a brown plant with a red cob ($A_1\ a_2\ B\ Pl\ P^{wr}$), therefore, the horny portions of the empty glumes were brown and the flowering glumes were red. The thin margins of the empty glumes were difficult to classify, but they appeared to be red similar to the flowering glumes.

A summary of the factorial relations of a_2 and p in relation to upper glume or chaff color is as follows:

$A_2\ P^{wr}$ = red chaff.
$a_2\ P^{wr}$ = red chaff.
$A_2\ p$ = white chaff.
$a_2\ p$ = white chaff.

Table 7 gives the data on four back-cross progenies which bear out this relationship. Inasmuch as these progenies also were back crosses for *Pl pl*, and as unfortunately the upper glume color of some of the purple plants could not be definitely established, it was necessary to group all the purple plants into one class.

TABLE 7.—*Data on progenies of the back cross* $\frac{a_2\ P^{wr}}{A_2\ p} \times a_2\ p$

Pedigree No.	Purple plants (A_2)	Sun-red plants (A_2)		Brown plants (a_2)		Green plants (a_2)	
		P^{wr}	p	P^{wr}	p	P^{wr}	p
7848	20	14	8	4	6	8	9
7849	11	10	9	6	12	8	11
7850	20	15	10	9	11	6	14
7851	14	13	13	8	6	8	4
Total	65	52	40	27	35	30	38
Expected	72	36	36	36	36	36	36
Deviation	−7	+16	+4	−9	−1	−6	+2

$\chi^2 = 11.62$. P = between 0.05 and 0.10.

Among the F_3 progenies grown there were a few that were homozygous $P^{wr}\ P^{wr}\ A_1A_1$ and that were demonstrated by appropriate tests to be segregating for A_2a_2. All the plants in these progenies had red upper glumes on their cobs (with the exception, of course, of the purple plants, which had considerable purple pigment in these parts). These segregations support the assumption that the upper glume color of $a_2\ P^{wr}$ plants is red.

A summary of the factorial relations of A_1, A_2, and P^{wr} in regard to upper glume color is as follows:

$A_1\ A_2\ P^{wr}$, $A_1\ a_2\ P^{wr}$ } red.
$a_1\ A_2\ P^{wr}$, $a_1\ a_2\ P^{wr}$ } brown.
$A_1\ A_2\ p$, $A_1\ a_2\ p$, $a_1\ A_2\ p$, $a_1\ a_2\ p$ } white or colorless.

Data supporting the foregoing relationship were obtained from some of the F_3 progenies. A summary of the data from two progenies segregating for these three factors is recorded in Table 8. The observed and expected numbers are not in very good agreement, but all of the expected classes are present.

TABLE 8.—*Data on two progenies by self-fertilization from plants of the composition* $A_1a_1\ A_2a_2\ P^{wr}\ p$

Item	Colored plants ($A_1\ A_2$)		Green plants ($a_1\ A_2$, $A_1\ a_2$, and $a_1\ a_2$)			Total
	Red chaff (P^{wr})	White chaff (p)	Red chaff (P^{wr})	Brown chaff (P^{wr})	White chaff (p)	
Observed	30	20	13	23	7	93
Expected	39.2	13.1	13.1	17.4	10.2	93
Deviation	−9.2	+6.9	−.1	+5.6	−3.2	0

χ^2=8.59. P=between 0.05 and 0.10.

TABLE 9.—*Data on a progeny by self-fertilization from a plant of the composition* $A_1a_1\ A_2a_2\ P^{wr}\ P^{wr}$

Item	$A_1\ A_2$ (red chaff)*	$A_1\ a_2$ (red chaff)	$a_1\ A_2$ and $a_1\ a_2$ (brown chaff)	Total
Observed	25	10	12	47
Expected	27	9	12	48
Deviation	−2	+1	−0	−1

* Also includes the purple plants with purple chaff.

A single F_3 progeny was obtained which was segregating for A_1a_1 A_2a_2 and was homozygous $P^{wr}\ P^{wr}$. A summary of the data from this progeny is recorded in Table 9. The agreement between the observed and expected numbers is very close in this case.

LINKAGE RELATIONS OF A_2a_2

Crosses have been made between a_2 and genes from 7 of the 10 linkage groups in maize. The linkage data from these crosses are summarized in Table 10.

Recombination values approximating 50 per cent were obtained between a_2 and all the factors with which it has been crossed except B. From the back-cross data on a_2 and B, crossover percentages of from 35.5 to 40.2 per cent may be computed, depending upon the

method used. The value of 37.2 ± 3.2 was computed from the first two classes. Gene a_2 also has been crossed with *lg*, and the small amount of F_2 data available indicate 55 ± 4.6 per cent of recombinations. These data come from three small progenies which, because of the extremely dry season of 1930, were all that were obtained.[7]

TABLE 10.—*Recombination percentages of a_2 with the genes with which it has been crossed*

Genes		Linkage phase [a]	$X\,Y$	$x\,Y$	$X\,y$	$x\,y$	Total	Recombinations
X	Y							
								Per cent
A_2	C	C *Bc*	4,666	←	14,422	→	19,088	[b] 51.1±0.3
A_2	R	C *Bc*	1,802	←	5,763	→	7,565	[b] 52.4± .6
B	A_2	C *Bc*	64	38	← 112 →		214	37.2±3.2
A_2 [c]	*Lg*	R F_2	92	35	74	34	235	55.0±4.6
A_2	*Y*	R *Bc*	617	631	573	635	2,456	51.0± .7
A_2	*Pl*	C *Bc*	118	123	124	93	458	53.9±1.6
A_2	Ts_2	R *Bc*	109	123	117	132	481	50.1±1.5
A_2	*Br*	R *Bc*	102	104	95	98	399	50.1±1.7
A_2	*Ra*	R *Bc*	231	359	298	359	1,247	47.3±1.0
A_2	*Ij*	C *Bc*	428	461	432	442	1,763	50.7± .8
A_2	A_1	R *Bc*	6,445	←	19,536	→	25,981	[b] 49.6± .3
A_2	A_1	C *Bc*	1,692	←	5,171	→	6,863	[b] 50.7± .6

[a] The symbols in this column have the following significance: C=coupling, R=repulsion, *Bc*=backcross progenies, and F_2=progenies by self-fertilization.

[b] Inasmuch as the recombination percentages in these distributions are computed from half of the total number of individuals, their probable errors also are based on the smaller numbers.

[c] A_1a_1 also segregating.

SUMMARY

A second pair of genes, A_2a_2, affecting anthocyanin pigment in maize has been isolated and its relation to some of the other aleurone and plant color genes studied.

The new factor pair is complementary to the A_1a_1, *Cc*, and *Rr* pairs in the production of aleurone color.

It is complementary to A_1a_1 in the production of plant color.

It differs from the A_1a_1 pair only in that it has no influence upon the color produced by the pericarp gene *P*.

Cob color appears to be dependent upon the color of two distinct parts of the cob, the lower glumes and the upper glumes.

The color of the lower glumes appears to be controlled by the plant color genes and not influenced by the pericarp gene, *P*. The color of the thin upper glumes or chaff is influenced by *P*, and it is the color of these parts that is not influenced by A_2a_2.

[7] Linkage tests conducted since this was written of a_2 with *lg*, gl_2, and r_1 indicate that a_2 is not in this group.

THRESHER INJURY IN BABY LIMA BEANS[1]

By H. A. Borthwick

Assistant Botanist, California Agricultural Experiment Station

INTRODUCTION

In the course of some germination studies of baby Lima beans, certain lots were found to produce rather high percentages of defective seedlings. The defects were of several different types, more than one of which frequently occurred in a single seedling. Of these various types of injury, one of the most conspicuous in the field when the beans are coming up is baldhead (fig. 2, D), a condition in which the stem growing point and the first true leaves of the plant are missing. Harter,[2] in a rather thorough study of the causes of this condition in many varieties of beans, found that, while insects and bacteria are partly responsible for the trouble, by far the greater amount is caused by the threshing machine. He also found that varieties differ considerably in the degree to which they may be injured by the thresher, Lima beans being rather susceptible. Harter did not mention any type of machine injury, however, other than baldhead. This paper will, therefore, describe in some detail these other types of thresher injury found in Lima beans.

TYPES OF INJURY

A study of many hundreds of baby Lima bean seedlings from machine-threshed seed shows that practically every part of the embryo is susceptible to some kind of thresher injury. In the following paragraphs, the various types of injuries are grouped according to the part of the plant affected.

INJURIES TO COTYLEDONS

Detachment of the cotyledons is one of the most common injuries. One cotyledon may be broken at its point of attachment to the stem, the other functioning normally (fig. 1, B); or both cotyledons may be absent although the plumule is uninjured (fig. 2, B and C). In either event, growth of the seedlings is retarded, although the retardation is much more pronounced in the latter than in the former case.

In many cases the cotyledon is not broken completely from the plant, but remains attached by a small area of tissue. Callus tissue develops at the broken surfaces, and not infrequently adventitious roots arise from this callused area of the cotyledon. In several cases cotyledons which had been broken off completely were found to have produced callus tissue and adventitious roots at their injured surfaces. (Fig. 3, B and C.) One cotyledon of this type was transplanted to a pot and grown for a few weeks. During this time the cotyledonary bud, which apparently in this case was broken off with the cotyledon, produced a branch an inch or more long, the roots made a vigorous growth, and the cotyledon was almost completely absorbed.

[1] Received for publication Oct. 5, 1931; issued May, 1932.

[2] Harter, L. L. Thresher injury a cause of baldhead in beans. Jour. Agr. Research 40: 371–384, illus. 1930.

Sometimes the fracture is of such a nature that, while the cotyledon still remains attached to the plant, its position with respect to the other cotyledon is altered. Such a situation is shown in Figures 1, A, and 4, C. One notes in the former case that the upper cotyledon, which is in its normal position, appears to be somewhat withered, while the lower one is still plump. In the latter case the opposite condition occurs, the upper cotyledon having been displaced. A break in the vascular connection of the plump cotyledon in each case probably explains why it has not lost its food reserve so rapidly as has the other one. Similar evidence appears in Figure 4, D, where a

FIGURE 1.—Lima bean seedlings showing injury to the cotyledons: A, Cotyledons at different levels as a result of injury near their point of attachment, the upper cotyledon being in its normal position; B, seedling with one cotyledon missing

cotyledon is shown with a crack crossing it transversely. Although the outer end is still attached, its food reserve has not been used as has that of the lower part of the cotyledon.

INJURIES TO HYPOCOTYL AND RADICLE

Besides the various injuries to the cotyledons just described, the hypocotyl and radicle are also subject to injury. Complete loss of the radicle is of comparatively frequent occurrence. (Fig. 4, C, D, and E.) In these cases the lower end of the hypocotyl calluses over and gives rise to adventitious roots. The radicle is occasionally found still attached to the lower end of the hypocotyl but injured to such an extent that it fails to grow. Although adventitious roots

soon replace it (fig. 3, D), the growth of the seedling is retarded in consequence of the injury. Damage to the radicle usually results in pronounced curvature near the point of injury. (Fig. 4, C, D, and E.)

A break in the hypocotyl frequently occurs just below the cotyledons, as in Figure 4, A and B. In this event the cotyledons remain below ground, while the first vegetative leaves reach the surface as a result of the elongation of the epicotyl. In these cases, adventitious roots also arise from the injured surface of the hypocotyl. Such seedlings are slow in coming up, and growers very often believe that they are from seeds which failed to get wet at the first irrigation.

A more common type of injury to the hypocotyl or root is one in which the fracture does not extend completely across. (Fig. 2, A, C, and D.) In a very large percentage of cases in which the hypocotyl

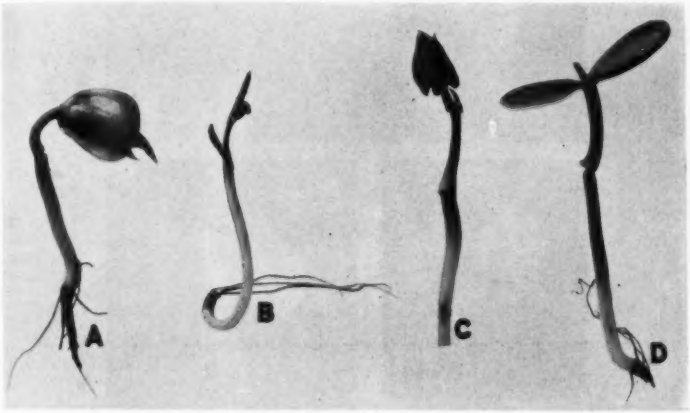

FIGURE 2.—Lima bean seedlings showing various types of injury: A, Crack in the hypocotyl; B, both cotyledons and the radicle missing; C, both cotyledons missing, and hypocotyl cracked; D, baldhead bean with cracked hypocotyl

is injured in this manner, the injury is on the side of the hypocotyl away from the seed coat. This condition is well shown in Figure 2, A, and also in Figure 5. The explanation of this phenomenon appears to be connected with the way in which the hypocotyl and root are supported in the seed. The root tip is completely enveloped by seed-coat tissue adjacent to the micropyle, which gives it rather rigid support, while the opposite end of the hypocotyl is supported by its attachment to the cotyledons. The middle part of the hypocotyl in a baby Lima bean lies along the edge of the bean, adjacent to the crack between the two cotyledons. Although it usually lies against the cotyledons, it is not very firmly supported by them. As this axis is well supported at each end and not in the middle, a blow on the hypocotyl, such as might be delivered by a cylinder tooth in a threshing machine, might reasonably be expected to result in a bending of this structure inward at that point and in the opening of a crack on the side of the hypocotyl opposite that which received the impact.

Injuries of the type just described are not so serious in their subsequent effect on the plant as are some of the others mentioned previously. In most cases healing of these cracks in the hypocotyl begins very shortly after the bean commences to germinate, and, by the time the plant has two fully formed true leaves, the wound is

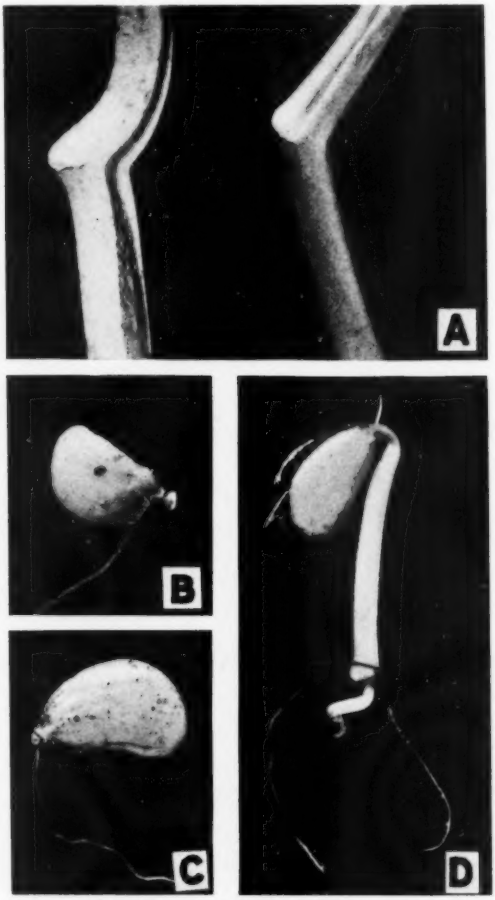

FIGURE 3.—Lima bean seedlings showing various types of injury: A, Cracked hypocotyls showing healing of the injury; B and C, detached cotyledon which has produced adventitious root from the wounded surface; D, seedling with injured radicle on which adventitious roots are being produced at the injured surface

completely healed. Early stages of such injuries are shown in Figure 5. These two embryos were taken from beans which had been soaked overnight. In later stages there is usually a sharp bend at the position of the injury, with an area of cork tissue forming a scar over the wound. (Fig. 3, A.)

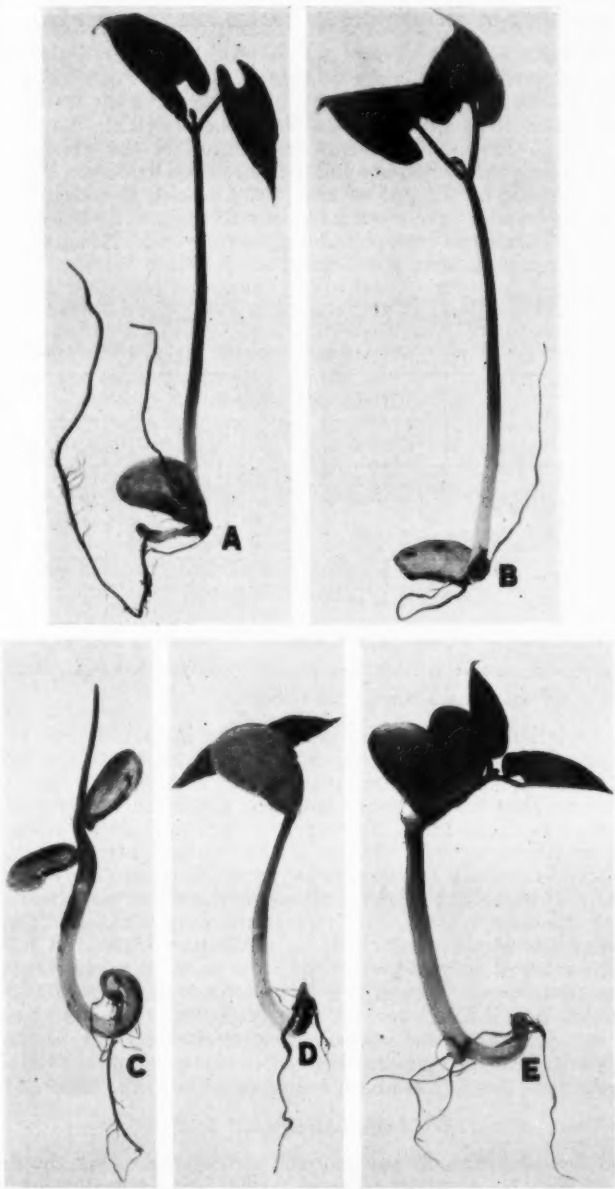

FIGURE 4.—Injuries to hypocotyl and radicle: A and B, Hypocotyl broken off at point of attachment of cotyledons, the hypocotyl and radicle being still present but not functioning in A; C, baldhead seedling with cotyledons at different elevations as a result of injury and with radicle missing; D, seedling with a fracture in one cotyledon, a crack across the hypocotyl, and the radicle missing; E, seedling with an injury to the hypocotyl near point of attachment of cotyledons, a transverse crack near the lower end of the hypocotyl, and radicle missing. Note the formation of adventitious roots at all injured surfaces

AMOUNT OF INJURY IN MACHINE-THRESHED BEANS

The relative amount of seed which produces injured seedlings of the types described in the preceding pages varies markedly in baby Lima beans from different sources. Twenty different lots of beans were obtained from growers near Sacramento, Calif., for germination studies. This material was germinated in the greenhouse in flats containing a rather coarse soil to insure good drainage. Observations were made at the end of about two weeks, at which time the primary leaves had nearly reached their full size and the cotyledons had not yet been entirely depleted of food reserves. Results obtained with these beans are shown in Table 1.

TABLE 1.—*Summary of germination results from 20 lots of baby Lima beans germinated in soil flats in the greenhouse*

[Figures represent percentages based on the average of three lots of 40 beans each]

Description of seedling	Percentage germination of beans in lot No.—																				Average germination
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
Normal [a]	63	75	65	57	55	43	85	55	70	76	63	53	59	71	54	25	84	72	77	94	64.8
Jointed hypocotyl [b]	8	5	14	19	21	28	5	7	7	10	15	12	14	14	9	26	2	0	1	1	10.9
Baldhead [c]	7	5	3	7	7	4	0	11	8	4	5	10	9	7	8	12	4	1	2	2	5.8
One cotyledon missing	7	2	7	6	5	11	4	8	8	2	5	12	7	4	8	5	2	14	11	2	6.5
Two cotyledons missing	2	2	3	1	2	0	0	2	0	0	0	2	0	1	1	1	2	0	0	0	1.0
Total seedlings	87	89	92	90	90	86	94	83	93	92	88	89	89	97	80	69	94	87	91	99	89.0
Ungerminated seed	13	11	8	10	10	14	6	17	7	8	12	11	11	3	20	31	6	13	9	1	11.1

[a] By a "normal seedling" is meant 1 with 2 cotyledons, 2 well-developed vegetative leaves, and an axis with no evidence of injury.

[b] "Jointed hypocotyl" refers to those seedlings in which the hypocotyl has been cracked but the wound has been healed.

[c] "Baldhead" refers to seedlings with the plumule missing.

In considering the amount of injury shown in these 20 lots of seed, one notes that, while baldhead is rather common, other types of injury occur much more frequently. In many cases these other injuries probably do not cause so much reduction in yield as does baldhead. In other cases, however, the injuries are so severe that the plants fail to survive. Many of the ungerminated seeds, it was found, failed to germinate because they were too completely shattered internally to make any growth, although there was no external evidence of this injury at the time the beans were planted. This was found repeatedly to be the case in seeds germinated on blotters, where they could be observed during the early germination stages. Accurate data concerning injury to underground parts of the seedlings grown in soil flats were not secured, because the seedlings were pulled out for inspection instead of being washed out of the soil. Had these injuries been accurately recorded, the number of seedlings showing defects would have been even greater than the table shows.

ARTIFICIALLY INDUCED INJURY

Hand-shelled beans do not produce seedlings showing the defects described above. A supply of hand-shelled beans was obtained from the field that produced lot 16 of Table 1. Lot 16 was machine threshed, and of the 20 lots investigated it produced the greatest number of defective seedlings. Several hundred of these hand-shelled

beans were germinated in soil flats in the manner already described. While a few cases of baldhead and of loss of cotyledons were observed, not a single seedling showed any injury to the hypocotyl or radicle.

In order further to establish the fact that the abnormalities found in bean seedlings were the result of rough treatment of the seed during threshing, attempts were made to produce the same effects artificially. For this work samples of hand-threshed beans were subjected to various types of mechanical injury. The beans of one lot were placed flat on the table and pressed until they could be heard to crack. In most cases no external injury to the seed coat was evident as a result of this treatment. The beans of another lot were placed on end in a small depression in a board, with the hilum directed to the front and the micropyle above. Each bean was then hit a sharp blow directly over the radicle and hypocotyl by the release of a steel spring. The beans were caught on cloth to prevent a second shock. Slight external injury resulted from this treatment. The beans showing injury to the seed coat were planted separately from the others.

These two lots of injured beans were germinated along with a lot of uninjured hand-shelled material. The uninjured seed produced normal seedlings except for a few cases of baldhead and loss of cotyledons. The seeds which had been subjected to pressure produced seedlings with a very high percentage of cracked and missing cotyledons, but practically no seedlings with injured hypocotyls or radicles. In the beans which were hit with the steel spring, there was a high percentage of injury to the hypocotyl or radicle. No significant difference appeared in this lot, however, between beans with external injury and those without. A few showed injuries to the hypocotyl similar to those of Figure 5, although in most cases the injury was so severe that the radicle and sometimes a part of the hypocotyl also were completely lost. In this lot of seedlings over 40 per cent showed some type of injury to the hypocotyl or radicle similar to injuries found in machine-threshed material. This observation seems to prove rather definitely that machine threshing is responsible for much of the injury found in bean seedlings.

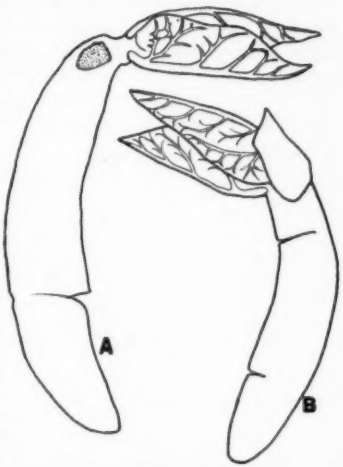

FIGURE 5.—Embryos dissected from soaked seeds. Cotyledons removed to expose injuries to hypocotyl. The embryo at A would probably give rise to a seedling similar to that of Figure 3, D, and the one at B, to one similar to that of Figure 4, D

SUMMARY

Baby Lima beans are found to be very susceptible to thresher injury. In addition to baldhead, which has been described by Harter and shown by him to be largely the result of thresher injury, the following additional types are here described and illustrated. (1) One or both cotyledons may be broken from the embryo; or, in other cases

where they still adhere to the plant, they may be injured to such an extent that the food reserves are not efficiently translocated. (2) Injury to the radicle results in its complete loss. In less severe cases it remains attached but ceases to function. Its place is taken by adventitious roots which arise from the injured areas. (3) The hypocotyl may be broken, in which case, if the break is near the top, the cotyledons remain below ground when germination occurs. Frequently the break is incomplete and heals as the seed germinates. This last type of injury does not materially retard the growth of the plant.

Injuries of the types described were found to be present in all lots of machine-threshed beans examined and very abundant in some of them. In hand-shelled beans, injuries to the radicle and hypocotyl were completely absent, and injuries to cotyledons and plumule were far less frequent than in machine-threshed beans. Injuries similar to those found in machine-threshed beans were produced in hand-shelled ones by subjecting them to mechanical shock before germination. The conclusion is drawn, therefore, that the defects found in baby Lima bean seedlings are the result of injury received in the threshing machine.

EFFECTS OF NUTRITION AND HEREDITY UPON LITTER SIZE IN SWINE AND RATS[1]

By H. P. MORRIS, *Research Assistant*,[2] *Division of Agricultural Biochemistry*, and DON W. JOHNSON, *Caleb Dorr Graduate Fellow, Division of Animal Husbandry, Minnesota Agricultural Experiment Station*[3]

INTRODUCTION

It is a common practice with the laymen working with multiparous animals to select breeding stock, both male and female, but more particularly female, from large litters in the belief that the factors governing size of litter are inherited by the progeny from their parents. It is of considerable economic importance to know whether or not litter size can be increased by the selection of breeding stock from the larger litters. If there are environmental factors which affect the size of litter, such, for example, as nutrition, then by improving these factors the breeder should be rewarded by an increase in the productiveness of his animals.

REVIEW OF LITERATURE

Rommel (*17*)[4] found the average litter size of Poland China sows for the period 1882–1886 to be 7.04 pigs and for the period 1898–1902 to be 7.52 pigs, an increase of 0.48 pig per litter. Rommel and Phillips (*18*) observed that the average litter size of 5-year-old Poland China sows was 8.40 pigs, while the average litter size of yearling sows was only 6.65 pigs. King (*11*) found that very young and very old female rats produced smaller litters as a rule than females of intermediate age. She concluded that both age and physical condition are important factors in the determination of litter size. Johansson (*9*) and Keith (*10*) in studies with swine, and Green (*5*) in studies with mice, among others, have also observed increase in litter size with increase in age of dam.

Wentworth and Aubel (*23*) found no difference in the average litter size of "big-type" and "small-type" Poland China swine. Their figures were 7.85 ± 0.05 and 7.89 ± 0.04, respectively. No data were given to show the actual difference in the size of the two types.

Hammond (*7*) concluded that the lower fertility of young sows is to a large extent due to the smaller number of ova shed, since he found the average number of corpora lutea in eight young sows to be 14.3 ± 0.39 and in nine old sows 19.77 ± 1.26. Loeb (*12*) found that when guinea pigs were underfed until they had lost up to 35 per cent of their body weight the Graafian follicles failed to develop or developed only partly, resulting in failure to ovulate. The underfeeding produced temporary sterility. He did not, however, report the

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[2] National Research Council Fellow.

[3] The authors wish to express their sincere appreciation to Dr. Leroy S. Palmer and Dr. Cornelia Kennedy for permission to use data obtained by them on litter size in rats, and to O. E. Mydland for help in keeping and tabulating the records of litter size in the small-animal breeding colony of the nutrition laboratory of the Division of Agricultural Biochemistry.

[4] Reference is made by number (italic) to Literature cited, p. 520.

length of time before normal ovulation recurred after normal feeding was resumed, Workers at the Cornell Agricultural Experiment Station (*14*) report the results of 20 years of selection in poultry. As a result of mating high-producing birds with high-producing birds on the one hand, and low-producing birds with low-producing birds on the other, two lines were developed showing marked differences in egg production.

Rommel and Phillips (*18*), in a study of litter size in Poland China swine reported a correlation coefficient of $+0.06 \pm 0.008$ between the size of the litters in which the mothers were farrowed and the size of litters farrowed by daughters of these mothers. The correlation coefficient decreased from $+0.108 \pm 0.014$ for yearling to $+0.032 \pm 0.037$ for 5-year-old daughters. Rommell concluded that there was a small but definite tendency for fecundity to be inherited, although its influence tended to be lost with increasing age. Johansson (*9*) found no significant correlation between two different litters of the same sow, but when he correlated the average of the first four litters with the average of the fifth to eighth litters, he obtained a correlation coefficient of $+0.468 \pm 0.07$. He also found a correlation coefficient of $+0.129 \pm 0.079$ when he correlated the average size of the first four litters of the mother with the average size of the first four litters of their daughters. From an analysis of data covering 35 years obtained at one of the largest pig-breeding stations in Sweden, Johansson concluded that the fertility of the sow is affected by environmental influences during growth and maturity. This explanation was made by Johansson to account for the variability in litter size which he found in his data but for which he was unable to account to an appreciable extent on the basis of heredity. Haines (*6*) obtained data on guinea pigs indicating that the environmental factors which influence size of litter are associated. Pearson and Weldon (*22*), correlating the size of litter of mother and daughter, concluded that in mice there is no evidence that litter size is inherited. Keith (*10*), working with 935 litters of seven breeds of swine, found no significant correlation in relation to its probable error between the size of one litter and the size of the succeeding litter when each breed was considered separately, but when all seven breeds were combined he obtained a correlation coefficient of $+0.34 \pm 0.03$ between the first and second litters and of $+0.367 \pm 0.04$ between the second and third litters. While these correlations appear to be statistically significant, they probably are of slight biological value because the large differences in litter size for the various breeds which were combined increase the length of the correlation surface and therefore increase the correlation coefficient. To be of biological value such comparisons should be confined to litters produced within a single breed unless it is first shown that there is no significant difference between the litter size of the breeds combined. It is possible that such a study as this, carried out under a carefully controlled environment, would give a different result.

Simpson (*20*) believed, from the results of a cross between a wild Schwarzwald boar and a Tamworth sow, that there was a definite tendency for litter size to be inherited as a dominant character. Wentworth and Lush (*25*) bred six Tamworth sows to a wild boar, and because the average litter size was 7.67 pigs as compared to 11 for the Tamworth breed, they concluded that the boar influenced the size of litter. Only 1 of the crossbred daughters reproduced, and she

produced but 1 litter, which consisted of 4 pigs. Since Wentworth and Lush found the result of their experiment in agreement with that of Simpson, they consider it suggestive of the dominance of the factors for wild litter size. Such results need to be verified by larger numbers, however, before the question is definitely answered.

Harris (*8*), in an analysis of data presented by Wentworth and Aubel (*24*), found a statistically significant correlation between the size of the litter in which a boar was farrowed and the size of the litters in which his daughters were farrowed. He also found a correlation of $+0.121 \pm 0.022$ between the size of the litters in which the grandsires and granddams were farrowed. Both these correlations are as large as the correlation which Wentworth and Aubel found between the size of the sow's litter and the size of the litter in which she was produced. There is no genetic reason for either of the two former correlations, hence one is led to question the source of the data from which the correlations were determined. Harris believed that such correlations might arise (1) through strains of animals of different breeders differing with respect to fertility, (2) through differences in the conditions under which breeders maintained their herds, provided such differences affected litter size, or (3) through actual dishonesty of certain breeders in reporting the size of litters for herd-book publication.

Buchanan Smith (*21*), from a review of the literature, reached the conclusion that litter size is definitely inherited as a comparatively simple Mendelian dominant, but says that perhaps hereditary factors are not as important in determining litter size as good husbandry and the mothering ability of the sow. His conclusions regarding the inheritance of litter size as a simple Mendelian dominant appears unwarranted in the light of the data at present available.

Evvard (*2*, *3*) and Evvard, Dox, and Guernsey (*4*) found that nutrition was an important factor in determining the size of pigs farrowed, but they did not obtain significant differences in litter sizes.

EXPERIMENTAL MATERIAL

This study was made in an effort to discover (1) what relation, if any, exists between the size of the litter of which the dam formed a part and the size of litter that she produced, and (2) to determine whether size of litter is affected by the nutrition of the dam. Accurate records from a rat colony maintained by the nutrition laboratory of the Division of Agricultural Biochemistry were available to the writers. This colony is kept in a well-lighted room the temperature of which is maintained between 75° and 80° F. throughout the year. The animals are fed a diet of natural foodstuffs designed to produce normal growth and reproduction (*15*). In addition to the records from these above animals, the authors were given access to data collected by L. S. Palmer and Cornelia Kennedy, from which it was possible to study the effects of a diet low in nutritive value upon the fertility of the dam.[5] Although the records were taken from an experiment planned for another purpose it is believed that they are entirely suitable for this study because of the accuracy with which they were

[5] This diet consisted of 310 parts cereal grains, 533 parts dextrin, 100 parts commercial casein, 50 parts timothy hay, and 0.3 per cent cod-liver oil. $CaCO_3$ and $Ca_3(PO_4)_2$ were included in the diet in such proportions so as to give certain degrees of acidity and alkalinity and certain percentages of calcium and phosphorus.

kept and because of the inbreeding that has been practiced. In this experiment the females were taken from the normal breeding colony. Most of them were virgin animals. They were placed on a special diet and mated to proven males so that the influence of the sire would not be a factor limiting size of litter.

A study of inheritance of litter size from an economic aspect was also made. Practically all the data for this study were taken from volumes 70 to 81, inclusive, of the herdbooks of the American Poland China Record Association, volume 81 being the last of the herdbooks obtainable at the Minnesota station. Most of the animals used in this study were born between 1918 and 1921. For a discussion of the accuracy of herdbook data the reader is referred to McPhee (*13*). He found that the herdbook data show fewer litters of 1 to 4 and 9, 11, and 12 pigs than did the experimental data, but the frequency of litters of 8, in the herdbook data, was almost double that in the experimental data. Assuming that all herdbook data are inaccurate and to the extent noted by McPhee, it may yet be said that the inaccuracies affect the litters in which the dams were produced to the same extent that they affect the litters produced by those dams, and probably would not, therefore, affect any correlation that might exist between the size of the dam's litter and the size of the daughter's litter.

LITTER SIZE IN RATS

The average size of litters for the various groups of rats was as follows:

	Average number of rats born per litter
Group 1. Litters raised on breeding colony diet; 364 dams from these litters were used in the experiments	8.75±0.09
Group 2. Litters produced by these dams (66 per cent first litters)	7.51± .10
Group 3. Only first litters from 329 dams whose age at birth of young ranged from 62 to 200 days	7.51± .10
Group 4. One hundred and sixty-two litters produced by dams kept on a diet of low nutritive value and sired by proven males (34 per cent first litters)	5.67± .15
Group 5. Litters produced by F_1 hybrids. The hybrids resulted from crossing two unrelated strains (18 litters)	11.06± .45

The difference between litter size, in the various groups, where $E_{diff.} = \sqrt{E_1^2 + E_2^2}$ is as follows:

Group 1 minus Group 2 $= 1.24 \pm 0.14$; Group 1 minus Group 4 $= 3.08 \pm 0.17$; Group 2 minus Group 4 $= 1.84 \pm 0.18$; Group 5 minus Group 1 $= 2.31 \pm 0.46$.

It will be observed that the average size of the dam's litter (8.75 ± 0.09) was significantly greater than the average size of litter produced by these dams (7.51 ± 0.10). This difference is due largely to the fact that a higher percentage of the progeny litters were first litters, as may be seen by comparing the average size of these litters (Group 2) with the average size of first litters (Group 3). The litter size is the same in both groups. If, however, the average litter size in Group 1 is compared with the average litter size in Group 4 (the group on a diet of low nutritive value) a difference of 3.08 ± 0.17 is found—a difference which is highly significant. Thus it is apparent that environment may prevent the genotype from expressing itself, for the females in these two groups were born from the same stock and

should have produced litters of the same size. There is also a significant difference between the average litter size of Groups 2 and 4. In Group 5, which consisted of litters obtained from F_1 females produced by crossing two unrelated strains of inbred rats, there is a significant increase in litter size, which must have come about through the influence of hybrid vigor.

Although Group 5 contained too few litters to make the difference observed between Groups 1 and 5 conclusive, the results do indicate the possibility of increasing litter size by crossing two inbred strains and following it by selection.

Figure 1 shows the correlation surface for the litter size of the dams (Group 1) when compared with the size of the litters which they pro-

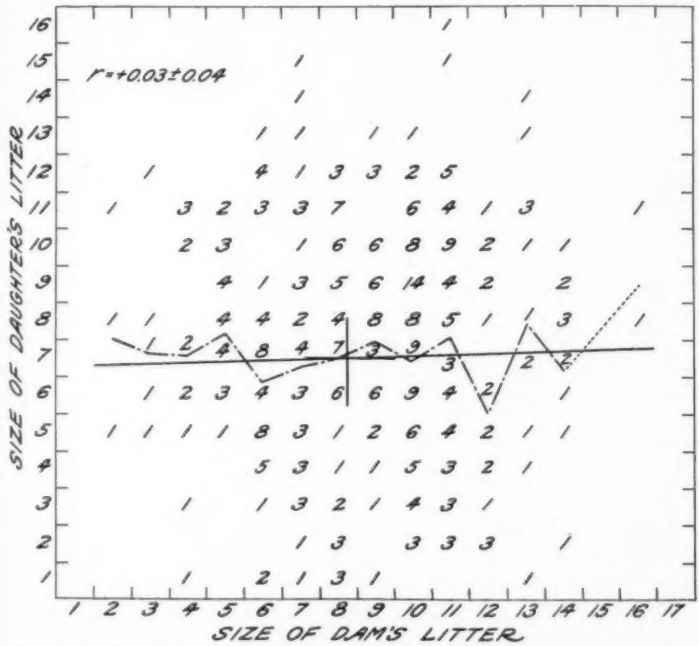

FIGURE 1.—Correlation surface showing relation between size of the litter in which the dam was born and size of the litter she produced, 364 rat litters having been used. The solid line represents the regression line and the dotted line the average size of the daughter litters for each litter size of the dam

duced (Group 2). The correlation coefficient for this figure is of the same order as its probable error, and is essentially zero. Figure 1 shows the high variability of litter size of the progeny from any class of mothers. It may be concluded from the data here shown that selection of dams from large litters has little influence upon size of litter in rats.

Various workers have found that there is an increase in litter size with the increase in age of the dam. Figure 2 shows a frequency surface for the size of first litter for female rats at various ages. These data give a correlation coefficient of $+0.10 \pm 0.04$, an insignificant

value for the numbers studied. This finding is in agreement with that of Johansson (*9*) in regard to the relation between age of dam and size of first litter in swine. To determine whether the size of the second litter of rats is related to the size of the previous litter, all the second litters, 74 in number, were correlated with the first litters. The resulting correlation coefficient was $+0.24 \pm 0.07$. While the correlation is not large and is not based on a large number of litters, it does indicate the possibility of increasing litter size by selection.

LITTER SIZE IN THE POLAND CHINA BREED OF SWINE

From the American Poland China Record 1,035 litters were selected at random. Most of these litters, it will be recalled, were born between

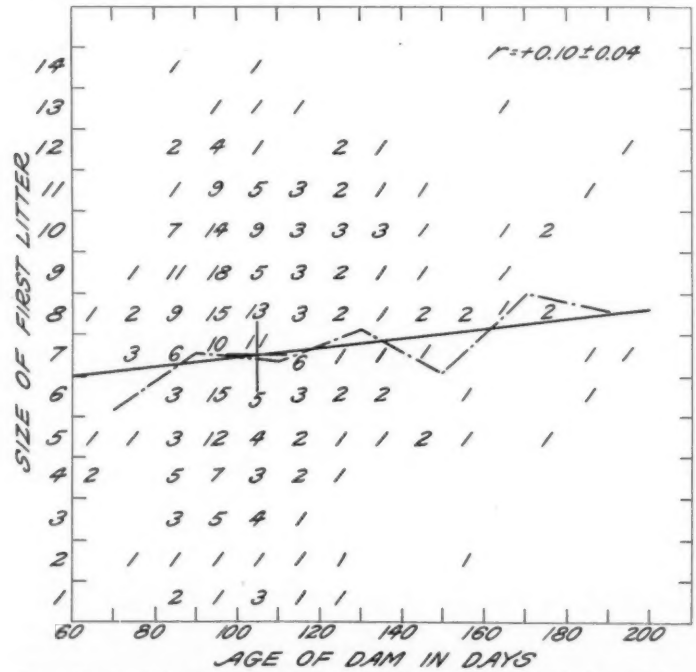

FIGURE 2.—Correlation surface showing relation between age of mother rat in days and size of her first litter. The solid line represents the regression line and the broken line the average size of litter for rats of different ages

1918 and 1921. The average size of the dam's litter was 8.69 ± 0.047, whereas the average size of 1,035 litters produced by them was 8.57 ± 0.048. A comparison of these values with those of Rommel (*17*) shows an increase of slightly more than 1 pig per litter during the time that elapsed between Rommel's investigations (1898–1902) and those herein reported (1918–1921). The difference in litter size of 1.05 ± 0.05 between these two groups is very significant. This difference is found to exist rather uniformly for all ages of the dam up to 60 months, as shown in Figure 3. It will be remembered that Rommel

found for the earlier period an average size of litter in this breed of 7.52 pigs. This was an increase of 0.48 pig per litter for the period 1898–1902 as compared to the period 1882–1886. The fact that there has been more than twice the increase in fertility in the Poland China breed from 1900 to 1920 than from 1880 to 1900 would indicate that the factors affecting litter size had had a greater influence during the former period than during the latter. This suggests the possibility that the change in type which occurred from 1900 to 1920 may have been a factor contributing to this increase in size of litter. It will be remembered, however, that Wentworth and Aubel did not find any

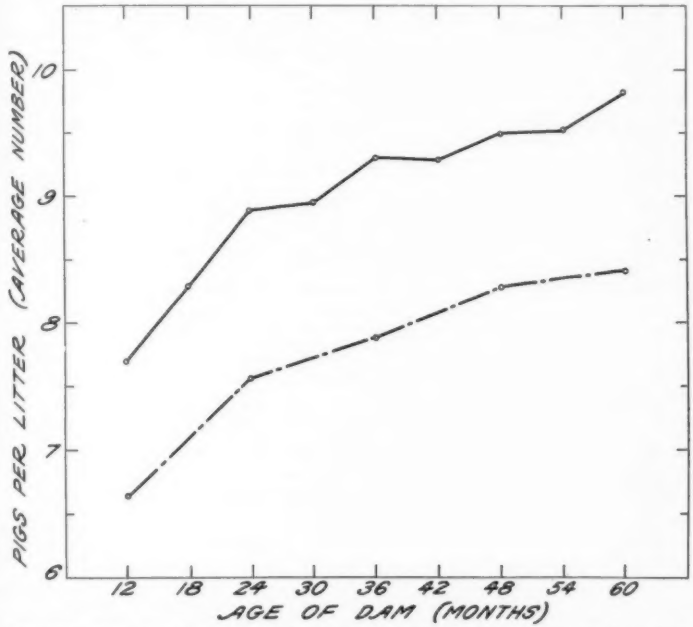

FIGURE 3.—Average number of pigs produced per litter by Poland China sows ranging in age from 12 to 60 months. The broken line represents results reported by Rommel and Phillips for the year 1902, the solid line those secured from herdbook records for litters born between 1918 and 1921

difference in litter size between the large and small type of Poland China swine.

A correlation coefficient showing the relation between the size of the dam's litter and the size of the litter which she would produce was found to be $+0.11 \pm 0.02$. There is no significant difference between this correlation and the correlation which Rommel and Phillips found in 1906 in the Poland China breed. From a study of the frequency surface for this correlation as shown in Figure 4, it may be concluded that the selection of dams from large litters would have but a slight effect upon the litter size of their progeny. Since Rommel and Phillips found a greater correlation coefficient for young dams and their progeny than for older dams and their progeny, it was decided that this correlation coefficient should be corrected for the influence of age of dam upon litter size. When this was done by the

partial correlation coefficient method, it was found that ${}_{a}r_{md} = +0.092 \pm 0.02$.[6] Only a slight decrease in the correlation coefficient was thus obtained when the age of the dam was made constant.

Since in Figure 3 there was a gradual increase in litter size with increase in age of dam up to 60 months, it was thought that there should be a fairly high correlation between age of dam and size of the litter that she would produce. This correlation was found to be $+0.31 \pm 0.02$, which is significant. These data, therefore, further

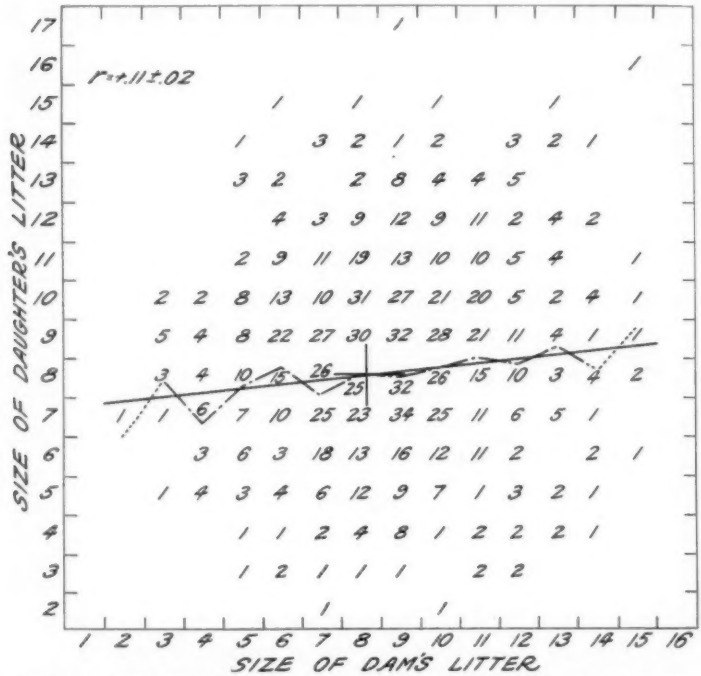

FIGURE 4.—Correlation surface showing relation between size of litter in which the dam was born and size of litter she produced, 1,035 litters of Poland China swine being represented. The heavy line represents the regression line and the dotted line the average size of the daughter litters for each litter size of the dam

verify the fact previously shown that there is a gradual increase in fertility of sows up to the age of 60 months.

Johansson (*9*) found a somewhat larger average litter size for sows farrowing their first litter at 14 to 16 months than at any other age up to 22 months, but considering the numbers with which he worked the differences he obtained were not statistically significant. The correlation between age of dam and size of first litter for 262 dams ranging in age from 10 to 15 months, inclusive, was also found in this study to be statistically insignificant. This is in agreement with Keith's (*10*) findings and also in agreement with the data for rats presented in Figure 2.

[6] a=age of dam, m=size of dam's litter, d=size of daughter's litter ${}_{a}r_{md} = \frac{r_{md} - r_{am} \cdot r_{ad}}{\sqrt{1-r^2_{am}} \cdot \sqrt{1-r^2_{ad}}}$

DISCUSSION

The number of animals born to a multiparous mother is influenced to a large extent by the physiological condition of the female before and at the time of oestrus. Haines (*6*) in studies of guinea pigs found that the major factors controlling litter size operate at conception. He found also that litter size is generally small from January to April, while from June to November it is unusually large. It seems probable that this difference is due largely to the nutrition of the mothers, for it is much easier to procure suitable green feed in summer than in late winter or early spring.

The size of litter depends primarily upon the number of ova which are released and which become fertilized. Parkes and Drummond (*16*) believe that any male capable of producing viable sperm is capable of fertilizing all of the ova produced. Warwick (*23*), in an examination of 3,967 fetuses, found 3.68 per cent in various stages of degeneration. It is not known, of course, to what extent heredity and environment, respectively, may account for this. There are many physiological conditions which affect the general health of the animal, and which would result in a smaller litter size. The data presented in this paper show that in the case of rats poor nutrition of the dam is one of the major factors affecting the number of young born per litter. It seems very probable, therefore, that improved feeding methods have influenced the measurable increase in litter size of the Poland China breed during the years 1885 to 1900 and 1900 to 1920.

Differences in litter size of breeds of swine have been definitely established by Bitting (*1*), Rommel (*17*), and Severson (*19*). Severson reported a litter size of 8.2 for Poland Chinas, Keith (*10*) found 7.91 during the period 1903–1925, Rommel reported 7.52 in 1906, and the present study shows 8.69. Such results as these suggest that there may be differences in litter size within a breed, an idea that is in keeping with the theory advanced by Harris (*8*) that strains of animals from different breeders may differ with respect to fertility. This difference, if it actually does exist, may not indicate any real difference in the fertility of the strain in question but may be explained upon a nutritional basis, that is, sows maintained at different locations and by different breeders may also be maintained on different planes of nutrition. This idea is supported by the studies of Johansson (*9*), who found that there had been no change in litter size at Bondeson's pig breeding station in Sweden for 35 years, where undoubtedly the best feeding practices were employed at all times.

If the size of litter is a valuable criterion in the selection of breeding stock, then the number in the litter of which the dam was a part should give an indication as to the size of litter that she will produce, and the size of the first litter should give an indication of the size of subsequent litters. The present study with rats and Johansson's study with swine show that the average size of litter produced by all individuals born in litters of any given size, as 10 for example (figs. 1 and 4), will be the average size of litter for the breed or species studied. The same is true of the size of second litters produced by dams all of which had produced the same size of first litters. This can be accounted for by environmental factors which affect litter size.

It does not, however, disprove the idea that litter size is or may be inherited, for it is well known that breeds differ with respect to litter size.

SUMMARY

A study of 1,035 litters of Poland China pigs, as derived from herd-book records, shows that there was an increase in average litter size amounting to one pig per litter in this breed between 1900 and 1920.

The swine data show an increase in average litter size with increase in age of dam up to 60 months.

The correlation coefficient between the size of litter in which the dam was born and the size of litter produced by her was very low in swine and essentially zero in 364 litters of rats.

The nutrition of the mother rat has a pronounced influence on the size of litter that she produces. Small litters result when the female is maintained on a poor diet.

The correlation coefficient obtained between age of dam and size of first litter in both rats and swine was found to be statistically insignificant.

The data used in this study show that, because of the influence of environmental factors on litter size, the size of the first litter is not an accurate indication as to what will be the size of subsequent litters. Consequently, while the data indicate the possibility of increasing litter size by selection, the size of the first litter should not be taken as the standard by which to select stock for breeding purposes.

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CORRELATIONS OF CERTAIN LINT CHARACTERS IN COTTON AND THEIR PRACTICAL APPLICATION[1]

By G. N. STROMAN

Associate Agronomist, New Mexico Agricultural Experiment Station

INTRODUCTION

Correlation is a tool that the plant breeder has long used in his efforts to breed desirable characters into new varieties. In relatively few instances, however, has correlation been of very great practical importance to plant breeders. In most cases the simple correlation coefficients alone, unless exceedingly high, have in themselves little value except as a means of calculating the partial, or net, and multiple correlations. For correlation coefficients to be reliable it is necessary that there be included in the calculations all measurable factors that might exert an influence on the particular character desired. When several characters or variables are used in the calculations it is not uncommon to obtain a high positive or negative simple correlation coefficient that will be entirely reversed when the partial or net correlation coefficient is computed.

This paper reports data on the relationships of percentage of lint, lint index, boll weight, and length of lint in certain related families of cotton. The simple and partial correlation coefficients between each of these characters are given, as are also the multiple correlation coefficients where each of the characters is used as the primary. The practical application of the data to breeding procedure is shown.

MATERIALS AND METHODS

In the spring of 1929 seed of a hybrid plant raised on the agronomy farm of the New Mexico Agricultural Experiment Station was planted under the number 179. The progeny segregated for practically all the characters of the cotton plant. Probably the hybrid was an upland-Egyptian cross, as it exhibited characters of both types. The families shown in Tables 1 to 3 were grown in 1930 from different individual plants of No. 179.

The plants grown in 1930 were harvested in the late fall after most of the bolls had opened, and the cotton was then ginned on a small 8-saw gin. The percentage of lint was calculated from the weight of clean lint and clean seed. The lint index was calculated from the formula: $\frac{\text{Weight of 100 seeds} \times \text{per cent lint}}{\text{Per cent seed}}$ (*8*).[2] Boll weight was calculated as the weight of lint in grams per boll. Length of lint was measured in sixteenths of an inch.

The correlations were calculated by the usual methods. The simple correlation coefficients were determined first and from these the multiple and partial correlations were obtained.

[1] Received for publication July 15, 1931; issued May, 1932.

[2] Reference is made by number (italic) to Literature Cited, p. 527.

THE DATA

The mean, standard deviation, and range are given in Table 1 for each of the characters for all the families. The simple and partial correlation coefficients between the four characters for each family are shown in Table 2, and the multiple correlation coefficients in Table 3.

LINT PERCENTAGE, LINT INDEX, AND BOLL WEIGHT

An examination of the partial coefficients shows that percentage of lint and lint index, as well as lint index and weight per boll, are significantly positively correlated; but the coefficients for percentage of lint and boll weight range from fairly positive to fairly negative.

TABLE 1.—*Range, mean, and standard deviation of percentage lint, lint index, boll weight, and length of lint of nine sister families of cotton*

Family No.	Number of plants	Percentage lint			Lint index		
		Range	Mean	Standard deviation	Range	Mean	Standard deviation
33	115	14–41	31.5±0.3	4.9±0.2	1.5–9.5	6.15±0.12	1.84±0.08
36	68	14–41	28.4± .5	5.9± .3	1.5–9.5	3.78± .13	1.64± .09
37	53	20–38	29.6± .4	4.2± .3	2.5–8.5	4.67± .15	1.60± .10
46	60	16–41	29.6± .5	5.2± .3	1.5–8.5	4.60± .15	1.77± .11
47	50	12–40	28.2± .6	6.1± .4	1.5–8.5	4.46± .19	2.02± .14
53	78	3–39	27.8± .6	8.5± .5	.5–9.5	5.08± .16	2.05± .11
60	85	23–39	31.4± .2	3.4± .2	3.5–9.5	5.77± .09	1.18± .06
69	77	12–42	33.4± .3	4.2± .2	2.5–9.5	5.95± .11	1.37± .07
94	127	6–42	30.4± .4	6.6± .3	.5–9.5	5.05± .12	1.93± .08

Family No.	Boll weight			Lint length		
	Range	Mean	Standard deviation	Range	Mean	Standard deviation
33	0–3.0	1.60±0.05	0.72±0.03	16–26	19.9±0.09	1.41±0.06
36	0–2.5	.82± .04	.51± .03	13–26	18.5± .2	2.7 ± .2
37	0–2.5	.92± .05	.54± .04	15–26	21.4± .2	2.5 ± .2
46	0–2.5	1.13± .05	.62± .04	15–24	19.1± .2	2.0 ± .1
47	0–2.5	.93± .6	.63± .04	16–23	18.8± .2	1.8 ± .1
53	0–3.0	1.33± .06	.73± .04	14–23	19.2± .1	1.7 ± .1
60	5–3.5	1.19± .04	.51± .03	15–22	19.1± .1	1.6 ± .1
69	5–3.0	1.48± .04	.52± .03	15–24	18.8± .1	1.7 ± .1
94	0–4.0	1.22± .04	.68± .03	14–27	20.8± .1	2.4 ± .1

TABLE 2.—*Simple and partial correlation coefficients between percentage lint, lint index, boll weight, and lint length in a series of related families of cotton*

Family No.	Number of plants	AB.CD [a]		AC.BD [a]		AD.BC [a]		BC.AD [a]		BD.AC [a]		CD.AB [a]	
		Simple	Net	Simple	Net	Simple	Net	Simple	Net	Simple	Net	Simple	Net
33	115	+0.76	+0.48	0.77	0.41	−0.37	−0.61	0.83	0.47	0.03	0.36	−0.04	0.17
36	68	.79	.63	.61	.20	−.45	−.51	.71	.41	−.19	.20	−.09	.16
37	53	.61	.46	.48	−.03	−.29	−.30	.76	.69	−.09	.19	−.17	−.15
46	60	.86	.55	.79	.24	−.11	−.01	.83	.56	−.03	.38	−.33	−.52
47	50	.80	.58	.67	−.04	.14	−.03	.86	.72	.21	.06	.21	.06
53	78	.91	.64	.85	.12	−.31	−.06	.90	.61	−.32	−.07	−.28	.01
60	85	.74	.72	.47	−.40	−.07	−.07	.84	.83	.03	−.04	.12	.13
69	77	.72	.30	.81	.62	−.16	−.38	.76	.38	.06	.13	.07	.26
94	127	.86	.76	.56	−.18	−.39	−.17	.74	.62	−.33	−.13	−.09	.21

[a] A, per cent lint; B, lint index; C, weight per boll; D, length of lint.

Griffee, Ligon, and Brannon (*3*) report simple high correlation coefficients between the above-mentioned characters similar in size and direction to those reported here. Their simple coefficient -0.872 ± 0.037, between number of bolls per pound and weight of 100 seeds was reduced in the partial correlation coefficient to 0.159 ± 0.151 when four other characters were held constant. The number of bolls per pound refers to boll weight, and the weight of 100 seeds to lint index, although they are not exactly the same.

Kearney (*5*), working with Pima cotton, obtained significant positive simple correlations between each pair of the three characters percentage lint and lint index, lint index, and boll weight, and percentage lint and boll weight.

The present writer (*10*), in a study of 7 varieties of cotton at the New Mexico station, obtained partial coefficients between percentage of lint and boll weight similar to those reported here. In 4 of the varieties fairly significant positive correlations were obtained, in 1 variety the correlation was barely significant (0.20 ± 0.07), in 1 variety it was not significant (0.06 ± 0.07), and in 1 variety it was negative (-0.25 ± 0.07).

Hodson (*2*) reported simple coefficients for lint percentage and boll weight, 1 negative (-0.45 ± 0.09), 1 positive (0.40 ± 0.06), and 3 that were not significant.

The genetic factors expressed in percentage lint and lint index, and lint index and boll weight seem to be the same; or, if different, they are closely linked. However, there are genetic factors other than these expressing themselves on each character, since the degree of correlation is not high enough to be explained on the basis of complete linkage of all the genes involved.

The relation of percentage of lint and boll weight may be explained on the basis of two linked genes with crossing over, the other genes being independent. Certain of these genotypes would segregate into a repulsion phase, others into the coupling phase, and still others into an independent phase in which only one of the linked genes would be present. In these nine sister families this could easily explain the range of the partial correlation coefficients from 0.62 to 0.40. Still, it seems logical to assume that if A and B [3] are correlated and B and C are correlated, A and C should be correlated. This was the case with the simple coefficients, but when the partial coefficients were obtained only three families were found to exhibit significant positive correlations. Genetically, then, there must be one gene of C linked with one of A that is not accounted for in B, whose true coefficient is covered up by the common association of the others.

LENGTH OF LINT AND OTHER CHARACTERS

In regard to length of lint and percentage of lint, most writers (*2, 3, 4, 5, 7, 9, 10*) have reported significantly negative correlations, but some writers (*4, 9, 10*) have found also a number of correlations that are not significant. Kottur (*6*), working with Indian cottons, reported independent inheritance in these two characters. In the nine sister families of the present study the correlation coefficients ranged from -0.61 to -0.01. The high negative correlation may be explained on the basis of two factors closely linked. One of these affects percentage of lint and the other length of lint. The coefficients showing no correlation may be explained as the expression of certain factors where not more than one of the linked genes is heterozygous.

The simple correlations of B and D are similar to the partial correlations of A and C, although the significant correlations are not so high. These simple correlations agree with those obtained by Kearney (*5*) on three populations of Egyptian cotton, where the coefficients were 0.360 ± 0.055, -0.132 ± 0.038, and -0.361 ± 0.085. However, the partials of B and D reported here show only two families with significant correlations and in no family was there a significant negative correlation. The genetic relationships of these two characters must be similar to those indicated for A and D, except that negative correlations between A and D were found, whereas the significant correlations between B and D were positive.

While the relation of C and D is similar to that of A and C, shown above, the one significant positive correlation is not very high, being only 0.26 ± 0.07.

MULTIPLE CORRELATIONS

The multiple correlations for the nine families are shown in Table 3. These coefficients vary greatly according to the family, as might be expected in progenies produced by different genotypes.

TABLE 3.—*Multiple correlation coefficients of percentage lint, lint index, weight per boll, and lint length when each is used as the primary*

Family No.	A.BCD	B.CDA	C.DCA	D.CAB	Family No.	A.BCD	B.CDA	C.DCA	D.CAB
33	0.88	0.87	0.86	0.62	53	0.91	0.95	0.91	0.32
36	.85	.84	.72	.61	60	.80	.93	.87	.19
37	.65	.82	.77	.34	69	.83	.78	.86	.39
46	.87	.91	.89	.54	94	.87	.92	.77	.43
47	.80	.91	.86	.22					

PRACTICAL APPLICATION OF DATA

In practical breeding procedure the partial correlation coefficients shown above are of some importance. Especially is this true of the relationship between lint percentage and length of lint. Cotton breeders have long recognized the negative correlation existing between percentage of lint and length of lint. Both of these characters are important to the commercial breeder because of the farmers' demand for a high percentage of lint and a good length of staple. The negative correlations obtained by most investigators have discouraged some breeders. Of course, if a breeder were selecting within a relatively pure strain for these particular characters and there was a negative correlation, it would be doubtful whether he could obtain both high percentage of lint and good length of staple, even with exceedingly large numbers. On the other hand, should there be no correlation, the maximum possibilities could easily be attained.

In regard to the relationship of percentage lint with boll weight, which certainly shows genetic correlations (*1*), three courses would be open to the breeder. In the case of the families showing positive correlations he would merely take for further testing those individuals with high percentage lint and high boll weight and the chances would be good that some would prove to be pure the following year for the characters desired. This would also be true for the families showing negative correlations, except that the individuals high in both characters would probably not be very numerous, and therefore a longer time would be necessary to obtain the desired characters. In the case of the noncorrelation families the matter is one of individual selection

and testing, which will require only fairly large numbers in the breeding program.

With respect to the three characters—percentage of lint, lint index, and boll weight—high simple correlations are obtained between each pair; so that in the families in which fairly high partial correlations are found between each pair of the three characters, a breeder using moderately large numbers would be justified in considering only one of the three, for by selecting for one he would automatically obtain, to a certain extent, the others as well.

SUMMARY

Data on nine sister families showing the correlation relationship between lint percentage, lint index, boll weight, and length of lint are reported.

The families studied sprang from a hybrid plant, which was probably an upland-Egyptian cross.

The mean, standard deviation and range are given for all four characters for each of the nine families.

Simple and partial correlation coefficients are shown between all characters for each family, and multiple correlations, using each character as the primary, are given for all families.

These data are discussed both from the standpoint of genetics and from that of the practical breeder. Lint percentage and length of lint are considered especially in relation to the other characters, and their practical importance is pointed out.

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A SIMPLE METHOD OF CONSTRUCTING TREE VOLUME TABLES [1]

By D. B. DEMERITT, *Associate Professor of Forestry*, and A. C. MCINTYRE, *Instructor in Forest Research, Pennsylvania Agricultural Experiment Station*

INTRODUCTION

A volume table shows for a given species the average contents of trees of different sizes (*5*).[2] In the past, most volume tables have been constructed by separating the field data or samples into diameter and height classes, plotting the class averages, drawing smooth curves and harmonizing them with each other.

Recently Reineke and Bruce [3] have referred tree volume to that of modified cylinders or frustums of ideal solids in the construction of alinement chart volume tables. Three factors, diameter, height, and form of tree, affect tree volume. Within any diameter-height group variations in form produce corresponding variations in volume. These three factors are harmonized through averaging, and the resulting table presents the assumed average trees for the universe from which the sample was taken.

If the sample is a true average for the whole and the mathematical and mechanical work of computation and curve construction have been carefully done, the other parts of the unit from which the sample came can be measured correctly by using the resulting tabular tree volume table or alinement chart volume table.

Bruce (*2*), Bruce and Reineke (*3*), and Reineke (*7*) have shown how alinement charts may be employed in the solution of other problems in forest mensuration. The development of the technic outlined in this paper was suggested by their articles.

Alinement charts have certain advantages over the older methods of volume table construction. Because of the fact that all of the data are used in the construction of a single curve, better curve definition results and fewer data are necessary. When data are deficient in a few diameter or height classes, the projection of the curve through these points, connecting with points having sufficient weight, is possible. It should be remembered, however, that usually the ends of the curves are defined by the smallest number of samples and extensions are open to error. Less time is required by the alinement chart method.

PROCEDURE

A knowledge of the several types of graph paper is assumed. In the preparation of alinement charts involving multiplication or division, some form of logarithmic paper should be used. The scales on such paper simplify the mechanical work of graduating the axes and make logarithmic computations unnecessary.

The present technic of volume table construction is based upon the use of log.-log. paper, since tree volume computation involves a multiplication. The equation of this multiplication is $y = ax^2$ $(h\ f)$,

[1] Received for publication July 27, 1931; issued May, 1932.

[2] Reference is made by numbers (italic) to Literature Cited, p. 539.

[3] REINEKE, L. H., and BRUCE, D. THE PREPARATION OF ALINEMENT CHART VOLUME TABLES. 1928. [Unpublished manuscript, Forest Service.]

in which y equals volume, a is a constant, x is the diameter, 2 is the exponent of x, h is height, and f is form factor (*5*). The substitution of different values of x in this equation, allowing height and form to remain constant, produces a parabolic curve. A parabolic curve on log.-log. paper plots as a straight line.

To simplify the explanation of the technic presented, there are included in this paper the average values from a sample of 209 red oak (*Quercus borealis maxima* M.) trees secured on five logging operations in central Pennsylvania. The data were collected in the usual manner (*1*), and the tree volumes were computed by cubing the logs according to Smalian's parabolic frustum formula. The d. b. h. (diameter at breast height measured at 4.5 feet above ground level) taken outside bark, is an average to the nearest tenth inch of two measurements taken at right angles to each other with tree calipers. Heights, in feet, were measured from the ground level to the tip of the main stem. The volumes shown are total volumes inside bark contained in the stem and limbs, in cubic feet. Volumes do not include stump. Utilization is to a 2-inch top inside bark.

The data, charts, and tables presented are used only for purposes of illustration. Additional samples for this species should be obtained if final charts and tables are to be constructed.

ARRANGING THE DATA

The trees are first classified and listed by d. b. h. height classes. Column totals for each class are then obtained. This method necessitates only one listing of the data and reduces computing time by nearly one-half. The units of classification are entirely arbitrary. In this case the d. b. h. classes used are 3.5 to 4.4 inches, 4.5 to 5.4 inches, etc., and the height classes are 30 to 39.9 feet, 40 to 49.9 feet, etc.

The data are then classified by d. b. h. classes regardless of height (Table 1, columns 3, 4, and 5). The totals found in the first classification are used, and the number of trees involved in each class are noted. The number of trees, d. b. h., height, and volume for each d. b. h. class are totaled and averaged.

In the same way, the trees are classified by height classes regardless of d. b. h. (Table 2, columns 2, 3, and 4) and the average total height and volume in each height class computed. The totals computed in the first classification are again used.

TABLE 1.—*Classification of trees by d. b. h. regardless of height*

D. b. h. class (inches)	Trees	Total d. b. h.	Total height	Actual tree volume	Form factor	Tabular volume
	Number	*Inches*	*Feet*	*Cu. ft.*		*Cu. ft.*
4	6	26.2	220.9	9.83		9.89
	4	16.9	180.5	7.42		7.41
	1	4.3	55.0	2.43		2.32
Total	11	47.4	456.4	19.68		19.62
Average		4.31	41.49	1.79	0.427	1.78
5	3	14.0	107.0	5.34		5.45
	3	15.9	157.5	8.98		9.59
	14	72.1	626.1	36.31		36.60
Total	20	102.0	890.6	50.63		51.64
Average		5.10	44.53	2.53	.400	2.58
6	2	11.8	75.6	4.93		5.90
	9	53.0	430.5	32.69		32.78
	4	24.9	213.5	18.95		18.23
Total	15	89.7	719.6	56.57		56.91
Average		5.98	47.97	3.77	.404	3.79

TABLE 1.—*Classification of trees by d. b. h. regardless of height*—Continued

D. b. h. class (inches)	Trees	Total d. b. h.	Total height	Actual tree volume	Form factor	Tabular volume
	Number	*Inches*	*Feet*	*Cu. ft.*		*Cu. ft.*
7	1	6.7	39.5	3.37		4.04
	5	34.5	226.0	20.42		23.52
	16	113.0	883.4	96.23		99.17
	5	36.5	325.2	37.25		40.12
Total	27	190.7	1,474.1	157.27		166.85
Average		7.06	54.60	5.82	0.393	6.18
8	1	7.6	37.5	4.42		4.95
	3	25.2	133.6	23.56		20.18
	17	135.1	940.6	133.74		134.22
	3	24.9	188.6	28.39		29.85
Total	24	192.8	1,300.3	190.11		190.00
Average		8.03	54.18	7.92	.415	7.92
9	3	27.6	146.4	30.25		27.55
	5	45.5	282.5	53.31		52.40
	6	55.3	374.8	71.03		71.60
Total	14	128.4	803.7	154.59		151.55
Average		9.17	57.41	11.04	.419	10.83
10	1	10.4	45.5	12.66		10.70
	13	131.5	742.0	170.64		166.20
	3	30.4	225.4	50.21		52.30
	12	120.3	746.6	162.31		164.90
Total	29	292.6	1,759.5	395.82		394.10
Average		10.09	60.67	13.65	.405	13.59
11	1	11.5	45.5	11.54		12.85
	3	32.8	169.7	43.97		43.15
	11	122.5	711.5	192.57		191.30
	2	21.5	143.8	32.18		36.95
Total	17	188.3	1,070.5	280.26		284.25
Average		11.08	62.97	16.48	.391	16.72
12	1	11.7	57.5	16.49		16.60
	7	83.5	455.0	137.09		138.50
	1	11.9	71.0	22.87		22.00
Total	9	107.1	583.5	176.45		177.10
Average		11.90	64.83	19.60	.392	19.68
13	1	12.6	52.0	16.19		17.50
	3	39.0	198.5	81.10		73.70
	4	51.9	302.1	116.14		113.40
	3	38.6	247.9	104.15		90.10
Total	11	142.1	800.5	317.58		294.70
Average		12.92	72.77	28.87	.436	26.79
14	3	41.8	200.9	76.16		90.30
	4	56.3	293.5	139.45		137.20
	5	70.1	412.7	182.99		186.10
Total	12	168.2	907.1	398.60		413.60
Average		14.02	75.60	33.22	.411	34.46
15	1	15.0	58.5	33.10		30.40
	1	14.6	68.8	39.45		34.30
	7	104.7	532.8	285.61		287.10
	1	15.0	85.0	48.16		45.00
Total	10	149.3	745.1	406.32		396.80
Average		14.93	74.51	40.63	.449	39.68
16	1	16.3	76.0	52.91		50.80
	2	32.2	162.5	115.19		103.50
Total	3	48.5	238.5	168.10		154.30
Average		16.17	79.5	56.03	.516	51.43
17	1	17.2	63.5	45.67		47.00
	4	67.8	291.8	194.02		211.40
Total	5	85.0	355.3	239.69		258.40
Average		17.0	71.06	47.94	.428	51.68
18	1	18.0	68.5	50.39		56.60
	1	17.9	89.0	77.67		69.00
Total	2	35.9	157.5	128.06		125.60
Average		17.95	78.75	64.03	.463	62.80
Grand total	209	1,968.0	12,262.2	3,139.73		3,135.42

TABLE 2.—*Classification of trees by height regardless of d. b. h.*

Height class (feet)	Trees	Total height	Actual tree volume	Tabular volume
	Number	*Feet*	*Cu. ft.*	*Cu. ft.*
30	6	220.9	9.83	9.89
	3	107.0	5.34	5.45
	2	75.6	4.93	5.90
	1	39.5	3.37	4.04
	1	37.5	4.42	4.95
Total	13	480.5	27.89	30.23
Average		36.96	2.14	2.32
40	4	180.5	7.42	7.41
	14	626.1	36.31	36.60
	9	430.5	32.69	32.78
	5	226.0	20.42	23.52
	3	133.6	23.56	20.98
	3	146.4	30.25	27.55
	1	45.5	12.66	10.70
	1	45.5	11.54	12.85
Total	40	1,834.1	174.85	172.39
Average		45.85	4.37	4.31
50	1	55.0	2.43	2.32
	3	157.5	8.98	9.69
	4	213.5	18.95	18.23
	16	883.4	96.23	99.17
	17	940.6	133.74	134.22
	5	282.5	53.31	52.40
	13	742.0	170.64	166.20
	3	169.7	43.97	43.15
	1	57.5	16.49	16.60
	1	52.0	16.19	17.50
	1	58.5	33.10	30.40
Total	65	3,612.2	594.03	589.88
Average		55.57	9.14	9.08
60	5	325.2	37.25	40.12
	3	188.6	28.39	29.85
	6	374.8	71.03	71.60
	12	746.6	162.31	164.90
	11	711.5	192.57	191.30
	7	455.0	137.09	138.50
	3	198.5	81.10	73.70
	3	200.9	76.16	90.30
	1	68.8	39.45	34.30
	1	63.5	45.67	47.00
	1	68.5	50.39	56.60
Total	53	3,401.9	921.41	938.17
Average		64.19	17.38	17.70
70	3	225.4	50.21	52.30
	2	143.8	32.18	36.95
	1	71.0	22.87	22.00
	4	302.1	116.14	113.40
	4	293.5	139.45	137.20
	7	532.8	285.61	287.10
	1	76.0	52.91	50.80
	4	291.8	194.02	211.40
Total	26	1,936.4	893.39	911.15
Average		74.47	34.36	35.04
80	3	247.9	104.15	90.10
	5	412.7	182.99	186.10
	1	85.0	48.16	45.00
	2	162.5	115.19	103.50
	1	89.0	77.67	69.00
Total	12	997.1	528.16	493.70
Average		83.09	44.01	41.14
Grand total	209	12,262.2	3,139.73	3,135.52

GRADUATING THE DIAMETER AXIS

On log.–log. cross-section paper, with the abscissa as d. b. h. and the ordinate as volume, the average values obtained in the second classification (Table 1) are plotted. A curve is fitted to the plotted values after proper weights have been assigned, as shown in the left-hand curve of Figure 1. It may be shown that the variation in the points from a smooth curve is due to differences in the height and form factor of the average tree. Column 6 in Table 1 shows the cylinder form factor of the average tree in each class. Figure 2 shows the

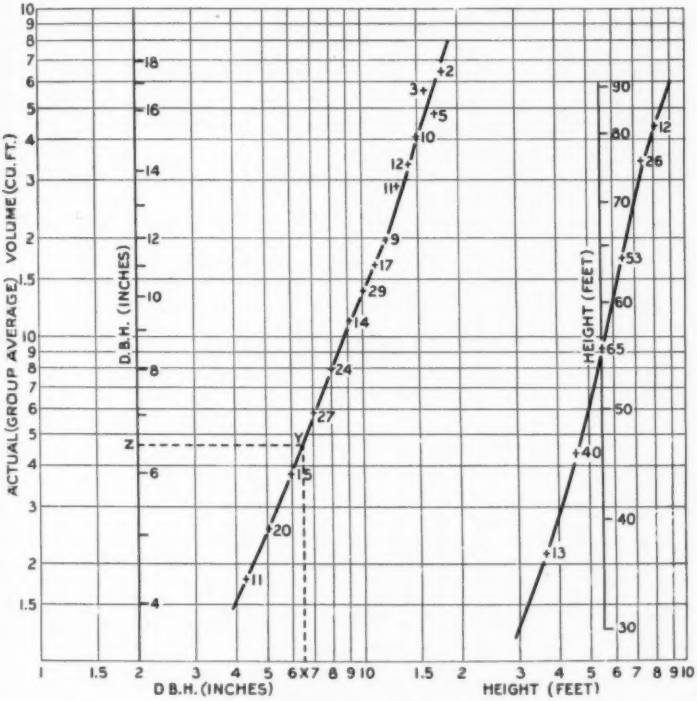

FIGURE 1.—Curves showing method of establishing diameter and height axes

extent of these variations and their relation to diameter. It is readily seen that slight changes in form and average height cause the plotted points to deviate from a smooth curve. The diameter classes above 12 inches show more radical changes in form factor, hence greater deviation from the trend of the other plotted values. In cases where more data are available the curve tends to become smoother owing to better sampling.

By using any convenient point near the left of the paper (in this case at abscissa value 2) the d. b. h. axis is established. If, now, the d. b. h. volume curve is used as a graduating curve, the graduations for d. b. h. may be placed on this axis. Even inches only are grad-

uated in Figure 1, to avoid confusion. The graduations are obtained by tracing vertically from the desired d. b. h. value on the abscissa to the graduating curve intersection, then horizontally to the axis. The dotted line (fig. 1) X–Y–Z shows the method of locating the 6.5-inch graduation.

GRADUATING THE HEIGHT AXIS

Utilizing the values obtained in the third classification (Table 2), a curve of volume on height is plotted. Volume is the dependent or ordinate value and height is the independent or abscissa value. Se-

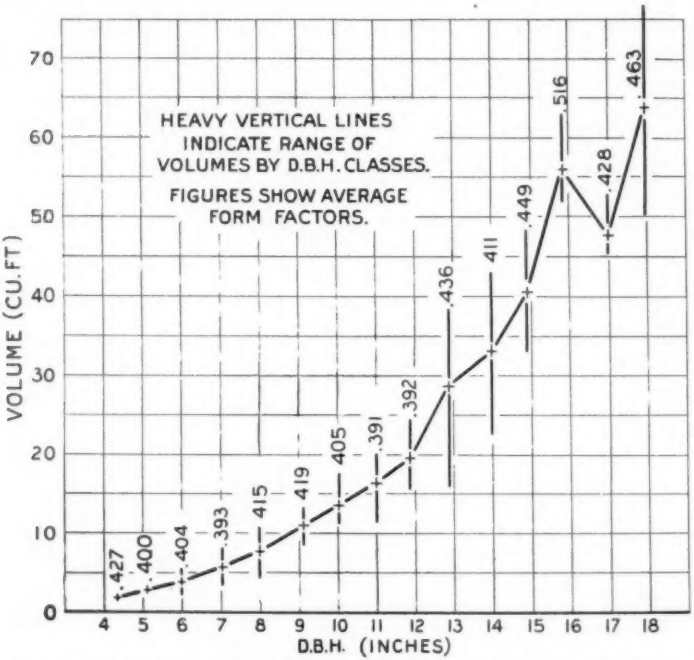

FIGURE 2.—Curve showing extent of tree volume variations and average form-factor values for each diameter class

lecting any convenient point near the right side of the graph paper, the height axis is set up. Using the height-volume curve as a graduating curve, the height values are placed on the height axis in the same manner as for the d. b. h. axis. Again, divergence of the plotted values from a smooth curve is due to fluctuations in the form factor of the average tree in the height classes.

LOCATING THE VOLUME AXIS

To locate the volume axis in cubic volume tables, any convenient d. b. h. and height may be assumed and the volume of a cylinder of these dimensions computed. Another cylinder 2 or 3 inches larger or smaller than the first is then assumed and the height necessary to pro-

duce a cylinder having a volume equal to that of the cylinder first assumed is computed. Construction lines between the d. b. h. and height values of these two cylinders are drawn, and the intersection of the two lines in the location of the volume axis. Repetition of this process for several assumed d. b. h. height values will show the exact position of the axis.

It will be found in this case that the volume axis is parallel to the d. b. h. and height axes and nearer to the d. b. h. than to the height axis. Its exact location depends upon the range of the d. b. h. and

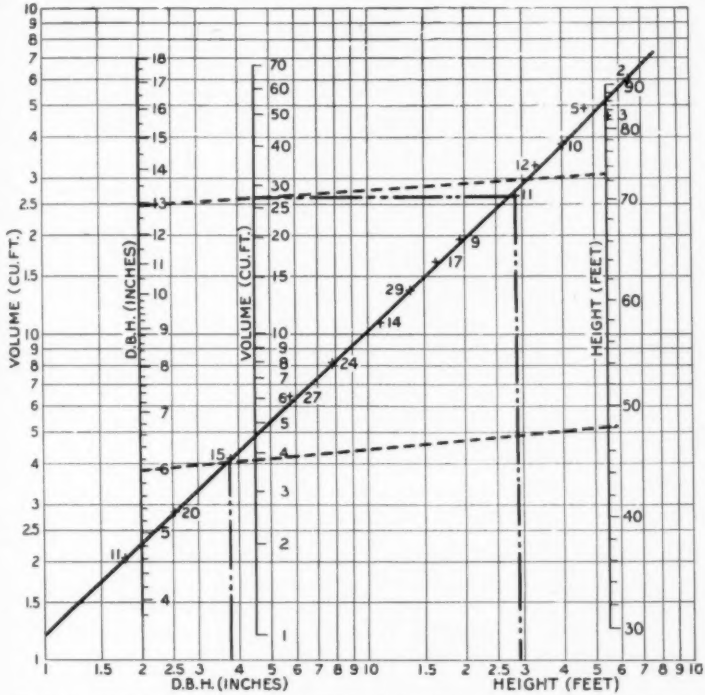

FIGURE 3.—Method of establishing values which locate the volume graduating curve. This drawing shows the form of an alinement chart volume table

height values and the spacing of their respective axes. A check of the location of volume axis is necessary in constructing a volume table.

GRADUATING THE VOLUME AXIS

Utilizing the average values in Table 1 (Columns 3, 4, and 5), place a straight edge on the d. b. h. and height values and mark the intersection on the volume axis, as shown by the dotted construction lines for the 6-inch and 13-inch d. b. h. classes in Figure 3. Using the abscissa as the actual tree volume, the average volume value is plotted on the abscissa horizontally opposite the intersection obtained by the pairing of the average d. b. h.-height values. The double dot and dash construction lines in Figure 3 show the method of locating the

volume values for the 6-inch and 13-inch classes, and these are respectively weighed with the number of trees in those classes.

A smooth curve is fitted to the plotted volume values thus obtained. It will be noted that the divergence from a straight line in these plotted values is less than in the case of the first curve, since d. b. h. is now associated with height, and the latter tends to influence the form factor in the reverse direction.

The volume graduations may now be placed on the volume axis, the volume curve being used as a graduating curve. In Figure 3, the major volume values are shown on the volume axis.

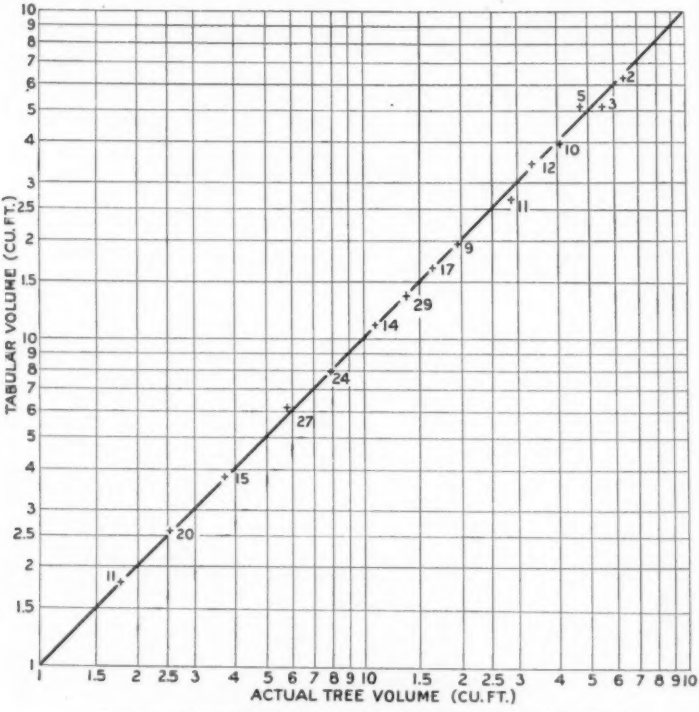

FIGURE 4.—Curve showing relation of tabular volume to actual tree volume

DETERMINATION OF ACCURACY

In order to check the accuracy of the graphic work (4) and to determine the limits of applicability of the table, it is necessary to read from the completed chart the volume of each individual tree used in its construction.

To determine the volume of any tree, a straight edge is laid on the chart intersecting the d. b. h. and height values on their respective axes. The volume of the tree is read at the point where the straight edge intersects the volume axis. These values are shown as tabular values in column 7 of Table 1.

For purposes of comparison with the actual tree volumes, the tabular values are totaled by classes. The grand total of the actual tree volumes is compared with the grand total of the tabular volumes. To compute the "aggregate percentage deviation" of the table, the difference between the actual and tabular volumes is obtained. This difference multiplied by 100 is divided by the total volume of the actual trees. A plus or minus sign is assigned to the result depending on the greater volume, tabular or actual.

The usual limit of accuracy for standard volume tables applicable over a large area is 1 per cent. In this particular case the aggregate percentage deviation is -0.134 per cent, which is well within the required limit.

A low aggregate deviation does not necessarily mean that the table as constructed is accurate, since too low volumes in the smaller diameters might offset too high volumes in the larger diameters, or vice versa. A graph of tabular volume (Table 1, column 7) plotted on actual tree volume (column 5) on log.–log. paper will plot as a straight 45-degree line through 1–1 provided the work is correct. Figure 4 shows a graph in which the plotted values are the averages by d. b. h. classes from Table 1.

Failure of these plotted values to produce a 45-degree line through 1–1 means that the volume axis should be regraduated in those portions as indicated by divergence from the 45-degree line.[4] This is accomplished by reading the ordinate volume value first and then the abscissa value from the curve as the corrected volume graduation. Reading of these corrected volume values at intervals depending upon their magnitude, will allow replotting over the abscissa values on the chart and a new volume graduating curve is produced. The volume axis may now be regraduated and the new individual tree volumes reread from the chart. The graduations on both the diameter and height axis should be carefully checked if considerable variation in tabular values is noted. Recomputation of the aggregate percentage deviation should produce a lower value.

The average percentage deviation is found by determining the percentage deviation of each individual tree volume from its chart volume. The total of these individual deviations taken without regard to sign, multiplied by 100, and divided by the number of trees, gives the average percentage deviation. The limit of this average deviation for standard volume tables should not exceed ± 10 per cent. The table here produced gives an average percentage deviation of ± 7.74 per cent.

PREPARATION OF THE FINAL TABLE

The volume table may be read from the alinement chart and tabulated in the conventional form. (Table 3.) Volumes are read for any desired d. b. h. and height interval in the same manner as explained above.

Using the alinement chart itself for determining tree volume makes interpolation unnecessary. For practical application it is, therefore, simpler to use the chart in its finished form rather than in the conventional table form.

[4] REINEKE, L. H., and BRUCE, D. Op. cit.

DISCUSSION

Previous alinement chart technic for volume table construction has utilized base charts for standard solid figures, cylindrical, parabolic or cone frustums, depending on the type of table desired. The technic has been based upon correlation of the tree volumes with these base charts. The technic here developed departs from previous practice in that base charts are not utilized.

Previous technic in volume table construction has first correlated the dependent variable, volume, followed by a fitting of the independent variables, d. b. h. and height. The present technic departs from previous practice again in that the independent variables are set up first, then the dependent variable, volume, is correlated with the two independents. Meyer (*6*) has simultaneously developed the same general principle of correlation but used a base chart with which to correlate first the independent, then the dependent variables.

Comparison of the present technic with previous technic, using identical data, indicates a saving of time in construction of graphs and in reduced correlations. In the comparisons made, the average percentage deviations obtained were in each case reduced by the present technic, the reduction ranging from 0.4 to 1.1 per cent.

TABLE 3.—*Merchantable volume[a] of red oak stand Pennsylvania, 1930*

D. b. h. (inches)	Volume (cubic feet) of trees of total height (feet) indicated							Basis number of trees
	30	40	50	60	70	80	90	
4	1.24	1.52	1.86					11
5	1.81	2.22	2.75					20
6	2.60	3.20	3.93					15
7	3.60	4.50	5.47	6.60				27
8	4.70	5.80	7.07	8.80				24
9		7.20	8.95	10.90	13.00			14
10		8.95	10.90	13.20	15.60			29
11		10.55	12.75	15.40	18.50			17
12			15.00	18.10	21.90	25.00		9
13			17.90	21.70	26.20	30.00		11
14			21.60	26.50	32.00	36.30	39.00	12
15			25.80	31.50	38.00	43.00	46.00	10
16				37.00	44.40	50.00	54.00	3
17				42.70	51.00	58.00	62.00	5
18				49.00	58.00	65.50	60.00	2
								209

[a] Volume includes stem and limbs inside bark above a 1-foot stump. Utilization limit is 2 inches inside bark. Heavy line indicates range of basic data. Aggregate percentage deviation: Table 0.134 per cent low. Average percentage deviation: ±7.74 per cent. Data collected in 1930 by A. C. McIntyre and T. A. Liefeld.

SUMMARY

A technic for the construction of alinement chart volume tables has been developed. Graduating curves for d. b. h. and height are plotted on log.–log. cross-section paper, and these two independent variables are then correlated with the dependent variable, volume, to produce the finished chart.

Except in the case of meager data, no axis regraduation is necessary since the initial graduations conform strictly to the variations in form factor of the trees measured.

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